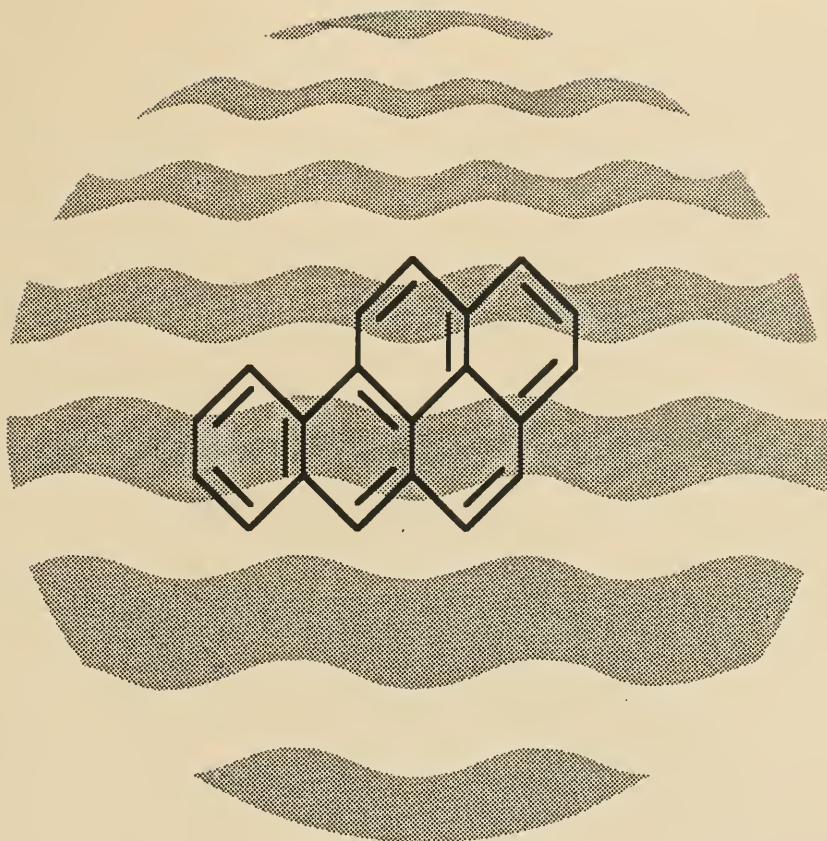


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THE ENVIRONMENTAL TOXICOLOGY OF POLYCYCLIC AROMATIC HYDROCARBONS

Prepared For:

THE ONTARIO MINISTRY
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THE ENVIRONMENTAL TOXICOLOGY
OF
POLYCYCLIC AROMATIC HYDROCARBONS

Report prepared for:
Hazardous Contaminants Coordination Branch

Report prepared by:
The Environmental Applications Group Ltd.

MARCH 1990



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EXECUTIVE SUMMARY

The objective of this project was to review the scientific literature pertaining to the effects of polycyclic aromatic hydrocarbons (PAH) on aquatic and terrestrial organisms. The studies were classified as primary or secondary, based on a set of criteria agreed upon earlier in the review process. Those studies which failed to meet these criteria were deemed secondary and of limited use in the standard setting process, but nevertheless useful in achieving a comprehensive review.

The report has been divided into two major sections addressing aquatic and terrestrial data. Within this framework, toxicity data was categorized as relating to either "lethal" or "sublethal" effects in order to facilitate the regulatory approach. Further infrastructure of the data recognizes the time frame over which the toxic impacts are realized, hence, the categories "acute", "subacute" and "chronic".

Ecological implications of aquatic toxicity

The data reviewed in this document represent only a fraction of the hundreds of known compounds in this group, and are therefore an incomplete database from which to generalize. However, most other PAH are either rare enough in the environment that no threat to aquatic life has been perceived or they are large and insoluble to the extent that negligibly low acute lethal toxicity may be assumed. For the PAH that have been tested, lethal concentrations are always orders of magnitude higher than concentrations found in even the most heavily polluted marine or freshwater environment.

However, an important concern associated with aquatic PAH contamination is the ecological significance of photo-induced toxicity. Numerous investigators, have documented toxic impacts mediated through this mechanism at solar ultra violet radiations intensities equivalent to or less than the maximum which could be expected in field situations. Photoactivation renders certain PAH toxics well within the confines of their solubility, and at concentrations which may occur in field situations. At this point, only a fraction of the known compounds have been tested for phototoxicity. It is therefore recommended that in developing an approach for the environmental regulation of PAH, consideration should be given to incorporation of phototoxicity data.

Conversely, heavily polluted aquatic sediments may contain PAH at concentrations equaling or exceeding levels acutely toxic to fish. Since many fish eggs incubate in stream-bottom gravel, and are considerably more sensitive to PAH than adults, there is a real possibility of exposure to toxic PAH concentrations.

Sublethal effects of PAH exposure have been more closely investigated than the lethal consequences particularly due to the high incidences of observed teratogenicity, mutagenicity and carcinogenicity in exposed organisms. Teratogenic effects often result in the death of newly hatched larvae and may significantly compromise their recruitment. The embryo-larval phase of fish is the most sensitive life-stage to PAH exposure.

Fish growth is not a sensitive indicator of chronic PAH exposure since other subacute effects are observed at the concentrations which influence growth. Therefore, evaluation of fish community health, with respect to contaminant exposure would likely be more effective if approached on the basis of genotoxicity, carcinogenicity and reproductive failure.

There is a limited quantity of information on the toxicity of PAH to freshwater invertebrates. Most of the information is of limited value since much of it is either based on marine studies or environmentally unrealistic concentrations. However, the chronic sublethal data clearly indicates that impact on fecundity is the most relevant concern. Since fecundity is an important factor in stabilizing lower trophic levels in the food chain, PAH contamination of the freshwater environment is expected to affect higher trophic levels.

Toxicity of PAH to algae is inadequately addressed in the literature. The major concern of algal exposure to PAH might be the ability of algae to bioconcentrate PAH and potentially enhance food chain transfer. This effect would amplify the exposure of herbivores.

Ecological implications of terrestrial toxicity.

In view of the limited database, extrapolation of the terrestrial toxicity data to predict ecological implications is somewhat difficult, and predominantly conjecture. Nevertheless, on the basis of the data examined, reproductive impacts to birds are highly likely and warrant further examination. Adducts formation with DNA of gametes may pose a serious problem from a population genetic perspective. This may be especially significant in female specimens since genetically damaged ova are not replaced as in the case of spermatozoa in males. However, additional research is required to reveal the ecological significance of these observations.

Finally, another potential threat to terrestrial herbivores is the ability of plant species to accumulate PAH in their tissues and hence increase the exposure of this group of animals to PAH toxicity.

Recommendations

In light of the literature reviewed, we consider that further development of the toxicity database is warranted, particularly with respect to fish since primary acute data are available for only six compounds. Simultaneously, thorough examination of the most common and priority PAH should be undertaken to establish which of these are phototoxic. Test organisms should include early-life-stages of fish which yield more sensitive data.

Sufficient data are presently available for developing an approach for the environmental regulation of PAH which should incorporate phototoxicity data in regulation elaboration.

Additional considerations in the regulatory approach are desirable for environments heavily contaminated with both PAH and PCB. The presence of PCB may enhance PAH uptake and favours metabolism of PAH to reactive intermediates.

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GLOSSARY OF TERMS

AH	Arylhydrocarbon (benzo[a]pyrene) hydroxylase
B[a]A	Benz[a]anthracene
B[a]P	Benzo[a]pyrene
B[b]kF	Benzo[b]k]fluoranthene
B[e]P	Benzo[e]pyrene
B[ghi]perylene	Benzo[ghi]perylene
BCF	Bioconcentration Factor
BCF _{mod}	Modified Bioconcentration Factor
BEND	Benzphetamine-N-demethylation
BPDE	Benzo[a]pyrene diol epoxide
BSR	(pmole B[a]P equivalents/g bile, wet wt.) / (pmole B[a]P/g sediment, wet weight).
C _b	Concentration of PAH in the biotic phase.
C _s	Concentration of PAH in the sorbed phase.
C _w	Concentration of PAH in the freely dissolved phase.
CBI	Covalent Binding Index
D[ah]A	Dibenz[ah]anthracene
DDT	4,4'-Dichlorodiphenyltrichloroethane
DMBA	7-12-dimethylbenz[a]anthracene
DHM	Dissolved Humic material
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
dsDNA	Double Stranded DNA
EC50	Concentration of a compound that produce an effect in 50% of exposed organism.
ECOD	Etoxicoumarin-O-deethylation
EROD	Etoxyresofurin-O-deethylation
ES	Lowest-Energy singlet state
EST	Singlet-triplet Splitting Energy
ET	Lowest Energy Triplet State
f	Fraction of total waterborne PAH bound to DHM.
GST	Glutathione-S-transferase
HPLC	High Performance Liquid Chromatography
IAA	Indole Acetic Acid
i.p.	Intra-Peritoneal
K'	Capacity Factor
K _b	Sorption Rate Coefficient of freely dissolved PAH to DOC
K _d	Depuration Rate Coefficient
K _{ow}	Octanol-Water Partition coefficient
K _p	Sorption Rate Coefficient of freely dissolved PAH to DHM
K _{rp}	Sorption Rate Coefficient derived through reverse phase separation.
K _u	Uptake Rate Coefficient
LC50	Concentration of a compound lethal to 50% of exposed organisms over a defined period of time.
LC100	Concentration of a compound lethal to 100% of exposed organisms over a defined period of time.

LD50	Dose of a compound lethal to 50% of exposed organisms over a defined period of time.
LOEC	Lowest Observable Effect Concentration
LSI	Liver Somatic Index
LSR	(pmole B[a]P equivalents/g liver, wet weight) / (pmole B[a]P/g sediment, wet weight).
LT50	Exposure Time required to yield 50% mortality of organism exposed to a defined toxicant concentration.
3-MC	3-Methylcholanthrene
MFO	Mixed Function Oxidase
MW	Molecular Weight
NOEC	No Observable Effect Concentration
PAH	Polycyclic Aromatic Hydrocarbon
PCB	Pentachlorophenol
p.o.	<u>per os</u>
QSAR	Quantitative Structure-Activity Relationship
SER	Smooth Endoplasmic Reticulum
ssDNA	Single strand DNA
SUVL	Solar UltraViolet Light
SUVR	Solar UltraViolet Radiation
TCDD	1,6[3H2]2,3,7,8-tetrachlorodibenzo-p-dioxin
TLC	Thin Layer Chromatography
TP	Phosphorescence Lifetime
TSI	Tissue Somatic Index
UV-LT50	LT50 derived through a phototoxic mode of action as influenced by Ultraviolet light during contaminant exposure.
WSF	Water Soluble Fraction

1. INTRODUCTION

The Ministry of the Environment is preparing a scientific criteria document for the development of multi-media standards for polycyclic aromatic hydrocarbon (PAH). Development of multi-media standards requires an integrated approach so that exposures to terrestrial and aquatic life forms in the Ontario ecosystem from all environmental sources (e.g. air, water, soil and diet) do not exceed an acceptable level.

The present document represents a comprehensive background report on the environmental toxicology of these compounds; in particular, aquatic animal toxicity, aquatic phytotoxicity, terrestrial animal toxicity and terrestrial phototoxicity and ecosystem effects.

When finally undertaking the study, the Ministry of the Environment identified 64 priority PAH to be addressed (Table 1.1). These compounds were investigated through several computer-aided literature searches, based on the nomenclature of the International Union of Pure and Applied Chemistry (IUPAC), common names, and also their Chemical Abstracts Service (CAS) number. These criteria were cross-referenced with various key words (Table 1.2) to extract specific toxicological information on these compounds. In addition to computer-aided searches, numerous major reviews were addressed along with discussions with various researchers in the field.

Through these efforts it has become apparent that the environmental PAH toxicological database exhibits numerous gaps and does not address many of the priority PAH originally identified by The Ministry of the Environment. Therefore, the adjusted research strategy was to address as much of the environmental toxicity data on any given PAH that could be retrieved. Hence, several PAH not cited in Table 1.1 have been addressed in the review.

TABLE 1.1
LIST OF PRIORITY PAH ADDRESSED
IN REVIEW OF ENVIRONMENTAL TOXICOLOGY

Acenaphthene	Coronene	Methylanthracenes
Acenaphthylene	Cyclopenta[cd]pyrene	Methylchrysene[1]
Anthanthrene	Dibenzothiophene	Methylchrysene[2,3,4 & 6]
Anthracene	Dibenzo[a,e]fluoranthene	Methylchrysene[5]
Benzo[a]fluorene	Dibenzo[a,e]pyrene	Methylfluorene[2]
Benzo[a]pyrene	Dibenzo[a,h]pyrene	Methylfluoranthene[2]
Benzo[a]fluoranthene	Dibenzo[a,i]pyrene	Methylfluoranthene[3]
Benzo[a]fluorene	Dibenzo[a,l]pyrene	Methylphenanthrene[3]
Benzo[a]chrysene	Dibenzo[a,i]carbazole	Naptho[2,3-b]pyrene
Benzo[c]fluorene	Dibenz[a,c]anthracene	Nitroanthracene[9]
Benzo[c]phenanthrene	Dibenz[a,h]acridine	Nitrobenzo[a]pyrene
Benzo[e]pyrene	Dibenz[a,h]anthracene	Nitrochrysene[6]
Benzo[ghi]fluoranthene	Dibenz[a,j]acridine	Nitrofluoranthene
Benzo[ghi]perylene	Dibenz[a,j]anthracene	Nitropyrene[1]
Benzo[g]chrysene	Dimethylphenanthrene[1,4]	Perylene
Benzo[j]fluoranthene	Dinitropyrene[1,6]	Phenanthrene
Benz[a]acridine	Dinitropyrene[1,8]	Phenylene-pyrene[ortho]
Benz[a]anthracene	Fluoranthene	Pyrene
Carbazole	Indeno[1,2,3-cd]pyrene	Tribenzo[aei]pyrene
Chrysene		Triphenylene
Benz[c]acridine		Dimethyl[8,9]benz-
Acridine		anthracene

TABLE 1.2
LIST OF GENERAL KEYWORDS CROSSREFERENCED
WITH CHEMICAL NAMES DURING COMPUTER AIDED
LITERATURE SEARCHES

GENERAL KEYWORDS		PHYTOTOXICITY
Accumulation	PAH	Absorption
Acute	Phycology	Adsorption
Amphibian	Plant	Algae
Aquatic	Polycyclic Aromatic Hydrocarbon	Apoplastic Transport
Bird	Polynuclear Aromatic Hydrocarbon	Bioaccumulation
Bioconcentration	QSAR	De Novo Synthesis
Burden	Review	Phytotoxicity
Carcinogen	Temperature	Protoplast
Chronic	Teratogen	Symplastic Transport
Crude Oil	Terrestrial	Visible Injury
Ecosystem	Transformation	Yield Reduction
Fish	Tumor	
Histology		
Invertebrate		
Marine		
Metabolite		
Mixture		
MFO		
Modifying Factor		
Mutagen		

The studies were classified as "primary" or "secondary", based on a set of criteria agreed upon early in the review process (Appendix I). These criteria were regarded as necessary for any study used in setting of environmental quality standards. Those studies which failed to meet these criteria were deemed secondary and of limited use in the standard setting process, but nevertheless useful in achieving a comprehensive review.

An additional limitation imposed in this review was the exclusion of experimental mammalian toxicology, since this was undertaken as a separate project by the Ministry of the Environment. In terms of terrestrial ecology, this exclusion of data significantly compromised the quality and volume of data remaining for terrestrial animals (e.g. wild and domestic animals, reptiles, amphibians, birds) as evidenced in Section 3 of this report.

The report discusses toxicological data under two broad categories. Chapter 2 addresses data pertaining to aquatic organisms, while Chapter 3 addresses data pertaining to terrestrial organisms. Within this framework, toxicity data were categorized under "lethal" or "sublethal" effects in order to facilitate a regulatory approach. Further infrastructure of the data recognizes the time frame over which the toxic impacts are realized, hence, the categories "acute", "subacute" and "chronic". In some situations, differentiation between acute and subacute was not easily made since the responses overlapped within the defined time frames. For example, some genotoxic effects were categorized over a time frame which spanned both acute and subacute categories. Where discrepancies of this nature occurred, the data were discussed under the single most appropriate category in order to facilitate the presentation of data reflecting a common mode of action.

Due to both the larger database available, and the inherent nature of aquatic toxicity, a discussion of the factors which modify the aquatic toxicity of PAH was warranted. This is presented in section 2.4 (modifying factors of toxicity).

A discussion of the toxicological data from the perspective of ecological implications is also presented for both the aquatic and terrestrial ecosystems (Section 2.5 and 3.7 respectively).

Finally, Chapter 4 provides a list of conclusions following the basic infrastructure of both the aquatic and terrestrial section. In addition, deficiencies in the toxicological database are noted and recommendations made.

2. AQUATIC TOXICOLOGY

2.1 FISH

2.1.1 Lethal Toxicity

Studies of acute and sub-acute lethality in fish are not numerous despite the long history of research on PAH. In fact, of all the studies documented here for freshwater fish, only two address compounds with more than three aromatic rings (pyrene and benzo[a]pyrene). Available data on acute toxicity are summarized according to primary and secondary studies (Table 2.1.1 and 2.1.2) as described in Appendix I.

2.1.1.1 Sources of Variation

Several sources of variability in toxicity should be noted at the outset. Because the aqueous solubilities of lipophilic PAH are so low, it is necessary to employ a carrier (usually methanol) so that concentrations high enough to cause mortality may be achieved. The carrier itself is not toxic at these concentrations, but it is not known what portion of the chemical in a suspension or in a colloidal state is available to test organisms, or how toxicity is affected (Holcombe et al. 1983).

Equally important from the standpoint of toxicity testing is the rapid decrease in volatility of PAH with increasing molecular weight. PAH solutions in water will decline in concentration through time, and low molecular weight PAH such as naphthalenes are particularly volatile. Consequently, static bioassays can produce badly inflated LC50 estimates because the actual concentration to which test organisms are exposed is much less than the nominal concentration and apparent toxicity is reduced. While this problem is well known to toxicologists, and is observed for other organic compounds, its severity for 2- and 3-ring PAH suggests that little reliance should be placed on static bioassays with these compounds unless in-tank concentrations are repeatedly measured.

TABLE 2.1.1
PRIMARY DATA OF ACUTE LETHAL TOXICITY OF PAHS TO FRESHWATER FISH

Chemical	Test Organism	Life Stage or Size	Method	Effect	Concentration (µg/L)	Temperature (°C)	Dissolved Oxygen (µg/L)	pH	References
Benaphthene	Brown Trout, <u>Salmo trutta</u>	0.16 g	FT,M,NA	96-h LC50	0.58	12.0	10.0-10.6	7.2-7.4	Holcombe <u>et al.</u> 1983
	Channel Catfish <u>Ictalurus punctatus</u>	5.0 g	FT,M,NA	96-h LC50	1.72	22.9	7.0-7.5	7.5-7.6	Holcombe <u>et al.</u> 1983
	Fathead Minnow <u>Pimephales promelas</u>	2 week post hatch	FT,M,NA	96-h LC50	0.61	24.5	7.9	7.4	Cairns & Nebeker 1982
	Fathead Minnow <u>Pimephales promelas</u>	0.16 g	FT,M,NA	96-h LC50	1.60	22.9	7.0-7.5	7.5-7.6	Holcombe <u>et al.</u> 1983
	Rainbow Trout <u>Salmo gairdneri</u>	1.3 g	FT,M,NA	96-h LC50	0.67	12.0	10.0-10.6	7.2-7.4	Holcombe <u>et al.</u> 1983
Atridine	Fathead Minnow <u>Pimephales promelas</u>	0.39 g	S,M,NA	96-h LC50	2.30 ^C	21.7	8.0-1.5 ^h	7.7-7.9	Blaylock <u>et al.</u> 1985
	Fathead Minnow <u>Pimephales promelas</u>	0.39 g	FT,M,NA	96-h LC50	2.90	20.3	8.1	7.6	Blaylock <u>et al.</u> 1985
	Fathead Minnow <u>Pimephales promelas</u>	0.27 g	S,M,NA	96-h LC50	2.24	20.0	4.3-8.5	7.8	Millemann <u>et al.</u> 1984
	Largemouth Bass <u>Micropterus salmoides</u>	Embryo	FT,M,A	<u>in ovum</u> LC50	10.50	20.2-23.2	7.1-8.4	7.1-8.4	Black <u>et al.</u> 1983
	Largemouth Bass <u>Micropterus salmoides</u>	Embryo-Larvae	FT,M,A	7-d LC50 ^a	1.02	20.2-23.2	7.1-8.4	7.4-8.1	Black <u>et al.</u> 1983
	Rainbow Trout, <u>Salmo gairdneri</u>	Embryo	FT,M,A	<u>in ovum</u> LC50	0.44	13.3-14.2	8.6-10.2	7.4-8.1	Black <u>et al.</u> 1983
	Rainbow Trout <u>Salmo gairdneri</u>	Embryo-Larvae	FT,M,A	27-d LC50 ^a	0.12	13.3-14.2	8.6-10.2	7.4-8.4	Black <u>et al.</u> 1983

TABLE 2.1.1 (continued)
PRIMARY DATA OF ACUTE LETHAL TOXICITY OF PAHS TO FRESHWATER FISH

Chemical	Test Organism	Life Stage or Size	Method	Effect	Concentration (µg/L)	Temperature (°C)	Dissolved Oxygen (µg/L)	pH	References
Anthracene	Bluegill Sunfish <u>Leponis macrochirus</u>	1.0-1.5g	FT,M,NA	96-h UV-LC50 ^d	0.005-0.046	22	8.2	7.1	Oris & Giesy 1986
	Bluegill Sunfish <u>Leponis macrochirus</u>	0.5-1.0g	FT,M,NA	96-h UV-LC50 ^e (1)	0.026	22	8.2	7.1	Oris & Giesy 1985
	Bluegill Sunfish <u>Leponis macrochirus</u>	0.5-1.0g	FT,M,NA	96-h UV-LC50 ^e (1)	0.003	22	8.2	7.1	Oris & Giesy 1985
	Bluegill Sunfish <u>Leponis macrochirus</u>	0.5-1.0g	FT,M,NA	96-h UV-LC50 ^e (2)	0.018	22	8.2	7.1	Oris & Giesy 1985
	Bluegill Sunfish <u>Leponis macrochirus</u>	0.5-1.0g	FT,M,NA	96-h UV-LC50 ^e (3)	0.012	22	8.2	7.1	Oris & Giesy 1985
	Bluegill Sunfish <u>Leponis macrochirus</u>	0.5-1.0g	FT,M,NA	96-h LC50 ^f	b	22	8.2	7.1	Oris & Giesy 1985
Fluorene	Bluegill Sunfish <u>Leponis macrochirus</u>	0.5-9g	S,N,NA	96-h LC50	0.91	22	--	7.2-7.4	Finger <u>et al.</u> 1985
	Fathead Minnow <u>Pimephales promelas</u>	<0.5g	S,N,NA	96-h LC50	>100.0	25	--	7.2-7.4	Finger <u>et al.</u> 1985
	Rainbow Trout <u>Salmo gairdneri</u>	0.3-.7g	S,M,NA	96-h LC50	0.82	12	--	7.2-7.4	Finger <u>et al.</u> 1985
Naphthalene	Fathead Minnow <u>Pimephales promelas</u>	0.27g	S,M,NA	96-h LC50	1.99	20.0	8.5-4.3 ^h	7.8	Millemann <u>et al.</u> 1984
	Largemouth Bass <u>Micropterus salmoides</u>	Embryo	FT,M,A	<u>in ovum</u> LC50	>0.24	20.2-23.2	7.1-8.4	7.4-8.1	Black <u>et al.</u> 1983
	Largemouth Bass <u>Micropterus salmoides</u>	Embryo-larvae	FT,M,A	7-d LC50 ^a	0.51	20.2-23.2	7.1-8.4	7.4-8.1	Black <u>et al.</u> 1983
	Rainbow Trout <u>Salmo gairdneri</u>	Embryo	FT,M,A	<u>in ovum</u> LC50	0.12	13.3-14.2	7.1-8.4	7.4-8.1	Black <u>et al.</u> 1983
	Rainbow Trout <u>Salmo gairdneri</u>	Embryo-larvae	FT,M,A	27-d LC50 ^a	0.11	13.3-14.2	7.1-8.4	7.4-8.1	Black <u>et al.</u> 1983

TABLE 2.1.1 (continued)
PRIMARY DATA OF ACUTE LETHAL TOXICITY OF PAHS TO FRESHWATER FISH

Chemical	Test Organism	Life Stage or Size	Method	Effect	Concentration (mg/L)	Temperature (°C)	Dissolved Oxygen (mg/L)	pH	References
Phenanthrene	Pathead Minnow <u>Pimephales promelas</u>	0.27g	S,M,NA	96-h LC50	b	20.0	8.5-4.3 ^h	7.8	Millermann <u>et al.</u> 1984
	Largemouth Bass <u>Micropterus salmoides</u>	Embryo	FT,M,A	<u>in ovum</u> LC50	>0.07	20.2-23.2	7.1-8.4	7.4-8.1	Black <u>et al.</u> 1983
	Largemouth Bass <u>Micropterus salmoides</u>	Embryo-larvae	FT,M,A	7-d LC50 ^a	0.18	20.2-23.2	7.1-8.4	7.4-8.1	Black <u>et al.</u> 1983
	Rainbow Trout <u>Salmo gairdneri</u>	Embryo	FT,M,A	<u>in ovum</u> LC50	0.04	13.3-14.2	8.6-10.2	7.4-8.1	Black <u>et al.</u> 1983
	Rainbow Trout <u>Salmo gairdneri</u>	Embryo-larvae	FT,M,A	27-d LC50 ^a	0.04	13.3-14.2	8.6-10.2	7.4-8.1	Black <u>et al.</u> 1983

Footnotes: a - exposure through egg stage to four days post hatch, terata also included as mortalities.
b - insignificant mortalities at saturation concentration.
c - estimated by a non-parametric method due to insufficient intermediate mortalities.
d - based on exposure time to solar ultraviolet "A" radiation (365 ± 36 nm) having a water surface intensity of 100 µW/cm² and intermittent light-dark regime.
e - based on continuous exposure to solar ultraviolet "b" radiation (310 ± 34 nm) with water surface intensity of (1) 14.8, (2) 70.0 or (3) 170 µW/cm².
f - based on exposures conducted under continuous darkness, cool-white fluorescent bulbs or gold fluorescent bulbs.
h - oxygen range from initiation to termination of exposure
FT - flow through bioassay
S - static bioassay
M - measured exposure concentrations
N - nominal exposure concentrations
A - aerated exposure vessel
NA - no aeration in exposure vessel

The primary data (Table 2.1.1) are limited to only 6 compounds, all of which have 3 aromatic rings or less. Acute toxicity to fish is generally in the range of 0.5 to 3.0 mg/L, although some exceptions exist. The first exception to note is that published for anthracene by Oris and Giesy (1986). Their toxicity data represent the photoinduced toxicity of this compound which is quite significant (96-h LC50 as low as 0.003 mg/L). Bowling et al. (1983) also exposed juvenile bluegills (Lepomis macrochirus) to anthracene in artificial outdoor channels (concentration at inflow: 0.0127 mg/L) and found rapid and complete mortality (<9 h) at the upstream end, but no mortality at the downstream end, where concentrations were lower.

However, by the morning of the second day, all remaining fish at the downstream end died also. Subsequent experiments confirmed that: (1) mortality was not due to anthracene alone (fish exposed in the dark all survived); (2) mortality was not due to photo-products in the water (shaded fish downstream of the unshaded, dosed region survived); (3) mortality was always due to photolysis of anthracene within the fish itself (fish dosed in sunlight all died). If fish were first exposed to anthracene, and then allowed to depurate the toxicant in the dark, subsequent mortality in sunlight was reduced (48-h depuration) or eliminated (144-h). The photoinduced toxicity of PAH is discussed in greater detail in Section 2.4 (Modifying Factors).

The remaining primary studies which address conventional acute toxicity suggest the embryo-larval stage is most sensitive to PAH exposure. In the case of phenanthrene, acute toxicity occurred in embryo-larval studies of rainbow trout at concentrations as low as 0.04 mg/L (Black et al. 1983). It should be noted that rainbow trout embryos were consistently more sensitive than largemouth bass embryos for acridine, naphthalene and phenanthrene (Black et al. 1983), which may reflect differences between the degree of yolk sac vascularization (and therefore contaminant uptake), lipid content, yolk-sac size, etc. of these cold and warm water species.

TABLE 2.1.2
SECONDARY DATA OF ACUTE LETHAL TOXICITY OF PAHS TO FRESHWATER FISH

Chemical	Test Organism	Life Stage or Size	Method	Effect	Concentration (mg/L)	Temperature (°C)	Dissolved Oxygen (mg/L)	pH	Reference
Acridine	Fathead Minnow <u>Pimephales promelas</u>	7-d Posthatch	SS,M,NA	UV-LT50	4.30/0.530 ^f	24	--	--	Oris & Giesy 1987
Acenaphthene	Bluegill Sunfish <u>Lepomis macrochirus</u>	0.32-1.2g	S,N,NA ^b	96h LC50	1.7	21-23	9.7-0.3 ^c	6.5-7.9	Buccafusco <u>et al.</u> 1981
Anthracene	Bluegill Sunfish <u>Lepomis macrochirus</u>	1.2g	FT,M,NA ^d	9h LC100	0.013	17	8.5	6.6	Bowling <u>et al.</u> 1983
	Fathead Minnow <u>Pimephales promelas</u>	7-d Posthatch	SS,M,NA	UV-LT50	15.75/0.005 ^f	24	--	--	Oris & Giesy 1987
	Fathead Minnow <u>Pimephales promelas</u>	0.8	S,M	0.5/24 LC50 ^a	0.36	14	--	--	Kagan <u>et al.</u> 1985
Benz[a]-anthracene	Fathead Minnow <u>Pimephales promelas</u>	7-d Posthatch	SS,M,NA	UV-LT50	65.09/0.002 ^f	24	--	--	Oris & Giesy 1987
7,12-dimethyl- benz[a]-anthracene	Guppy <u>Poeciliopsis</u> sp.	<2 weeks	Se	20h LC100	0.50	--	--	--	Schultz & Schultz 1982
Benzanthrone	Fathead Minnow <u>Pimephales promelas</u>	7-d Posthatch	SS,M,NA	UV-LT50	0.83/0.050 ^f	24	--	--	Oris & Giesy 1987
Fluoranthene	Bluegill Sunfish <u>Lepomis macrochirus</u>	0.32-1.2g	S,N,NA ^b	96h LC50	3.98	21-23	9.7-0.3 ^c	6.5-7.9	Buccafusco <u>et al.</u> 1981
	Fathead Minnow <u>Pimephales promelas</u>	0.8g	S,M	0.5/24 LC50 ^a	0.20	14	--	--	Kagan <u>et al.</u> 1985
Naphthalene	Coho Salmon <u>Oncorhynchus kisutch</u>	0.3	FT,M	96h LC50	3.22	--	--	--	Moles 1980
	Coho Salmon <u>Oncorhynchus kisutch</u>	1.0	FT,M	96h LC50	2.1	8.0	--	7.0-9.0	Moles <u>et al.</u> 1981
1-chloro-naphthalene	Bluegill Sunfish <u>Lepomis macrochirus</u>	0.32-1.2g	S,N,NA	96h LC50	2.3	21-23	9.7-0.3 ^c	6.6-7.9	Buccafusco <u>et al.</u> 1981

TABLE 2.1.2 (continued)
SECONDARY DATA OF ACUTE LETHAL TOXICITY OF PAHS TO FRESHWATER FISH

Chemical	Test Organism	Life Stage or Size	Method	Effect	Concentration (mg/L)	Temperature (°C)	Dissolved Oxygen (mg/L)	pH	Reference
Octachloro-naphthalene	Bluegill Sunfish <u>Leponis macrochirus</u>	0.32-1.2g	S,N,NA ^b	96h LC50	>600	21-23	9.7-0.3 ^c	6.5-7.9	Buccafusco <u>et al.</u> 1987
Pyrene	Fathead Minnow <u>Pimephales promelas</u>	0.8g	S,M	0.5/24 LC50 ^d	0.22	14	--	--	Kagan <u>et al.</u> 1985
	Fathead Minnow <u>Pimephales promelas</u>	7-d Posthatch	SS,M,NA	UV-LT50	3.20/0.026 ^f	24	--	--	Oris & Giesy 1987
Benzo[a]-pyrene	Fathead Minnow <u>Pimephales promelas</u>	7-d Posthatch	SS,M,NA	UV-LT50	40.05/0.006 ^f	24	--	--	Oris & Giesy 1987

Footnotes: a - phototoxicity bioassay, photosensitization (h)/observation period (h)
b - toxicant incompletely dissolved
c - dissolved oxygen range given from initiation of exposure to termination after 96h
d - phototoxicity bioassay, conducted in outdoor stream channel in sunlight
e - bioassay conducted in dark to circumvent phototoxicity
f - LT50 (hr)/concentration (mg/L), UV intensity: 100 uW/cm² (UV-A), 20 uW/cm² (UV-B)
FT - flow through bioassay
S - static bioassay
SS - semi static bioassay
M - measured exposure concentrations
N - nominal exposure concentrations
NA - no aeration in exposure vessel

Although previous studies have reported trends such as increasing toxicity with increasing molecular weight, aromatization and hydrophobicity (Milleman 1984, Neff 1985, Eisler 1987), it is difficult to support this on the basis of the limited freshwater fish data. Even consideration of the secondary acute data (Table 2.1.2) is of limited value in discerning trends in toxicity because of the highly variable techniques and conditions employed during the tests (e.g. static vs. flow through exposure, temperature, nominal vs. measured concentrations, phototoxicity).

In order to provide some means of comparison, these compounds were ranked according to the lowest concentration (mM) which elicited acute lethalties, regardless of test specimen, technique and temperature. Photo-induced toxicity data were not included in this ranking. On the basis of primary studies, the following order of compounds reflects increasing acute lethal toxicity: fluorene < acenaphthene < acridine < naphthalene < phenanthrene. When the secondary studies are also considered, the order of increasing acute toxicity is octachloronaphthalene < fluoranthene < 1-chloronaphthalene < fluorene < acenaphthene < acridine < 7,12-dimethyl-benz[a]anthracene < naphthalene < phenanthrene. This ranking of compounds does not readily support previously reported trends of increasing toxicity with increasing molecular weight, however, it admittedly suffers from some inconsistencies between studies.

Naphthalene, the second most acutely toxic compound, is of particular importance because of its toxicity and solubility. Naphthalene is the simplest (two unsubstituted aromatic rings) and most soluble (31.7 mg/L, Finlayson-Pitts and Pitts 1986) PAH. It also constitutes a substantial portion of the lightweight water soluble aromatics found in crude oils (NRCC 1983) and was previously reported as a PAH of moderate toxicity, on the order of 2 to 9 mg/L (Moles 1980; Moles et al. 1981; U.S.EPA 1980b). However, the present review recognizes acute toxicity towards rainbow trout embryo-larvae at concentrations as low as 0.11 mg/L (Black et al. 1983).

Additional evidence, although circumstantial, also suggest higher molecular weight compounds are limited in acute toxicity. The results of various studies in which PAH injections were used to induce mixed function oxygenase activity in fish are summarized in Table 2.1.3. With the exception of one study (Singh et al. 1985), all studies maintained injected fish for at least 72 h, and typically for 96 h or longer. None of these studies cited mortalities as a result of the injected dose. While some of the doses are quite impressive (e.g. 300 mg/kg, B[a]P, Gerhart and Carlson 1978), it should be noted that the typical route of administration was intraperitoneal, using an oil carrier. Under these circumstances, there would be some delay time for the PAH to migrate from the carrier and enter circulation. In the case of B[a]P (Gerhart and Carlson 1978), the measured tissue concentration (whole-body minus viscera) in rainbow trout, 3 and 5 days following injection of 300 mg/kg, was considerably lower than the injected dose (3.26 and 0.46 mg/kg wet wt, respectively).

2.1.1.2 Chronic Lethalities

Except for the work with fish eggs mentioned earlier, there have been few reports of long term lethal toxicity. Brown et al., (1977, cited in Neff, 1979) exposed bluegill sunfish (Lepomis macrochirus) to 1.0 mg/L benz[a]anthracene, and found 87% of the fish died within 6 months. Also, working with bluegills, Finger et al. (1985) reported only 12% mortality from a 30-d exposure to 0.50 mg/L fluorene, about half of the acute lethal concentration. These results are essentially extrapolated short term toxicity data, and indicate that PAH, like the majority of toxicants, have an asymptotic relationship between lethal concentration and exposure time. Hence, the 96-h LC50 is close to the ultimate LC50 when exposure time is extended indefinitely.

TABLE 2.1.3
SELECTED STUDIES IN WHICH INTRAPERITONEAL INJECTIONS
OF VARIOUS PAH AND DOSES DID NOT RESULT IN ACUTE LETHALITIES

Chemical	Test Organism	Dose (mg/kg)	Carrier	Exposure Time(h)	Reference
Benz[b]anthracene	Rainbow Trout (<u>Salmo gairdneri</u>)	10	Corn oil	96	Stathan <u>et al.</u> 1978
Benzo[b]triphenylene	Little Skate (<u>Raja erinacea</u>)	3 x 10	Corn oil	240	Bend <u>et al.</u> 1979
3-methylcholanthrene	Carp (<u>Cyprinus carpio</u>)	20	?	36	Singh <u>et al.</u> 1985
	Rainbow Trout (<u>Salmo gairdneri</u>)	20	Corn oil	96	Stathan <u>et al.</u> 1978
	Rainbow Trout (<u>Salmo gairdneri</u>)	2 x 20	Peanut oil	336	Forlin 1980
	Sheepshead minnow (<u>Archosargus probatocephalus</u>)	2 x 20	Corn oil	216	James & Bend 1980
	Stingray (<u>Dasyatis sabina</u>)	3 x 20	Corn oil	432	James & Bend 1980
Phenanthrene	Atlantic cod (<u>Gadus morhua</u>)	100	Soyabean oil	96	Goksoyr <u>et al.</u> 1986
	Atlantic cod (<u>Gadus morhua</u>)	50 ^a	Capsule	72	Goksoyr <u>et al.</u> 1986
Benzo[a]pyrene	Rainbow Trout (<u>Salmo gairdneri</u>)	300	Peanut oil	120	Gerhart & Carlson 1978

Footnote: a - oral dose

2.1.1.3 Mixture Toxicity

Undoubtedly, the most typical field exposure to PAH will entail some degree of mixture toxicity. Studies of this nature are even more limited than those conducted on pure compounds (Table 2.1.4). In addition, studies which address PAH mixtures invariably include additional non-PAH compounds which further complicate the problem of identifying mixture toxicity.

Woodward et al. (1987) addressed the acute toxicity of the water soluble fraction of several shale oils to coho salmon, cutthroat trout and fathead minnows. The toxicity (96-h LC50) of the shale oils ranged from 1.3 to greater than 5.0 mg/L. This toxicity is no worse, and in many cases, is actually less than many of the purified compounds listed in Tables 2.1.1 and 2.1.2. Cutthroat trout were typically the most sensitive of the three species tested. The most toxic response resulted from exposure of cutthroat trout to Paraho crude (96-h LC50 = 1.3 mg/L). However, comparison of the composition of this water soluble fraction to those of other crudes tested does not indicate any unusual level of constituent which could account for this response.

Additional studies conducted by Eastmond et al. (1983), on solvent refined heavy distillate, also indicate acute toxicity within the same range as that noted for shale oils. Palawski et al. (1985) reported the toxicity to striped bass of a customized mixture which was suggested as a reference standard. The reliability of the reported toxic concentrations, however, is suspect because of the static nature of the test and the use of nominal concentrations. On the basis of the reported mixture, it is most likely that the metals rather than PAH were the primary toxic species.

TABLE 2.1.4
SECONDARY DATA OF ACUTE LETHAL TOXICITY OF PAH MIXTURES TO FRESHWATER FISH

Industrial Mixture	Test Organism	Life Stage or Size	Method	Effect	Concentration (ng/L)	Temperature (°C)	pH	Comments	Reference
Shale Oils ^a									
Geokinetics(Ge) (Crude)	Coho Salmon	12.0g	FT,M	96-h LC50	4.5	20	8.4	composition of each exposure regime	Woodward et al. 1987
	Cutthroat Trout	1.1g	FT,M	96-h LC50	1.8	10	7.5	represents the water soluble fraction of	
	Pathead							a 100 mg/L (oil/water) mixture	
	Minnow	0.2g	FT,M	96-h LC50	2.9	20	8.4		
Tosco (To) (Crude)	Coho Salmon	3.1g	FT,M	96-h LC50	2.3	20	8.4		
	Cutthroat Trout	0.8g	FT,M	96-h LC50	2.1	10	7.5		
	Pathead								
	Minnow	0.2g	FT,M	96-h LC50	2.3	20	8.4		
Paraho (Pa) (Crude)	Coho Salmon	2.3g	FT,M	96-h LC50	4.2	20	8.4		
	Cutthroat Trout	1.5g	FT,M	96-h LC50	1.3	10	7.5		
	Pathead								
	Minnow	0.2g	FT,M	96-h LC50	>1.7	20	8.4		
Paraho HDT (PH)	Coho Salmon	4.4g	FT,M	96-h LC50	>2.4	20	8.4		
	Cutthroat Trout	0.9g	FT,M	96-h LC50	>5.0	10	7.5		
	Pathead								
	Minnow	0.4g	FT,M	96-h LC50	>2.4	20	8.4		
Paraho JP-4 (PJ)	Coho Salmon	3.7g	FT,M	96-h LC50	>2.1	20	8.4		
	Cutthroat Trout	1.9g	FT,M	96-h LC50	>1.5	10	7.5		
	Pathead								
	Minnow	0.2g	FT,M	96-h LC50	>2.1	20	8.4		

TABLE 2.1.4 (continued)
SECONDARY DATA OF ACUTE LETHAL TOXICITY OF PAH MIXTURES TO FRESHWATER FISH

Industrial Mixture	Composition (mg/L)	Test Organism	Life Stage or Size	Method	Effect	Concentration (mg/L)	Temperature (°C)	pH	Comments	Reference
Solvent Refined Heavy Distillate (Coal Liquifaction Product)	5-6% Aliphatics 59-69% PAH+PASH ^b 17-21% PAH+PANH ^c 9-14% HPAH ^d	Channel Catfish, <u>Ictalurus punctatus</u>		FT,M	96-h LC50	1.68	--	7.4	-Log K_{ow} =3.53 -acetone carrier used in all assays	Eastmond <u>et al.</u> 1983
		Cutthroat Trout, <u>Salmo clarki</u>	Embryo	FT,M	120-h LC50	2.31	--	7.4		
		Rainbow Trout <u>Salmo gairdneri</u>		FT,M	96-h LC50	1.58	--	7.4		
Customized Synthetic Mixture	e	Striped Bass <u>Morone saxatilis</u>	35 (post hatch)	S,N	96-h LC50	e	20.0	7.9	-acetone carrier used for organics	Palawski <u>et al.</u> 1985

Footnotes: a - Composition of shale oils (mg/L):

	Ge	To	Pa	Ph	Pj
Aliphatics	5.2	0.33	1.6	18.5	14.5
Aromatics					
Monoaromatics	3.7	2.9	0.38	8.8	8.5
Diaromatics	0.98	0.30	0.08	0.05	0.66
3 ring-aromatics	0.02	<0.01	<0.01	0.07	<0.01
Aliphatic					
Ketones	<0.003	0.74	<0.003	<0.003	<0.003
Phenols	4.6	2.0	1.9	<0.002	<0.002
Pyridines	2.8	1.8	1.0	<0.002	<0.002
Indoles	0.92	0.42	0.32	<0.003	<0.003
Quinolines	<0.002	0.32	<0.002	<0.002	<0.002
Alkylthiophenes	0.06	0.35	0.19	<0.001	<0.001

b - Polycyclic aromatic sulfur heterocycles

c - Polycyclic nitrogen heterocycles

d - Hydroxylated polycyclic aromatic hydrocarbons

e - Numbers represent the concentration (µg/L) of each component of the LC50 mixture: Toxaphene 0.018; Kepone 0.104; Aroclor 1248 0.216; Aroclor 1254 0.216; Aroclor 1260 0.216; Chlordane 0.035; DDE 0.018; Perylene 0.288; Fluorene 0.288; Phenanthrene 0.288; Pyrene 0.288; Benz[a]anthracene 0.288; Chrysene 0.288; Lead 7.19; Arsenic 7.19; Selenium 14.38; Copper 7.19; Cadmium 21.60.

FT- flow through bioassay

S - static bioassay

M - measured exposure concentrations

N - nominal exposure concentrations

2.1.2 Sublethal Toxicity

2.1.2.1 Acute Effects

Sublethal acute effects of PAH exposure to fish were not regarded to be substantially different from subacute effects which are addressed in the following section. Indeed, several responses which would technically be viewed as acutely sublethal (i.e. occurring in less than 4 d) such as teratogenesis and genotoxicity, were also well documented as subacute studies (i.e. effects occurring following a 7-d exposure). In the case of teratogenesis, some test species possess a very short embryonic phase over which exposure is only feasible, and are by definition acute. However, for the purposes of maintaining continuity, such effects have been addressed under the complimentary subacute sections.

Effects which are clearly acute were reported by Oris and Giesy (1986) for bluegill sunfish exposed to anthracene in the presence of UV radiation. He noted a four fold increase in ventilation rate within a 24 h exposure to 0.03 mg/L anthracene (Figure 2.1.1). Stressed and dead fish showed signs of asphyxia (open mouth, splayed opercula and pale gill filaments). The gross indicators of respiratory distress were also supported by histological examinations which revealed an erosion of the gill epithelium. Structural disorganization and erosion of epithelium also resulted on the dorsal surface and resembled a sunburn effect. These signs are consistent with free radical membrane erosion mediated through photoinduced toxicity.

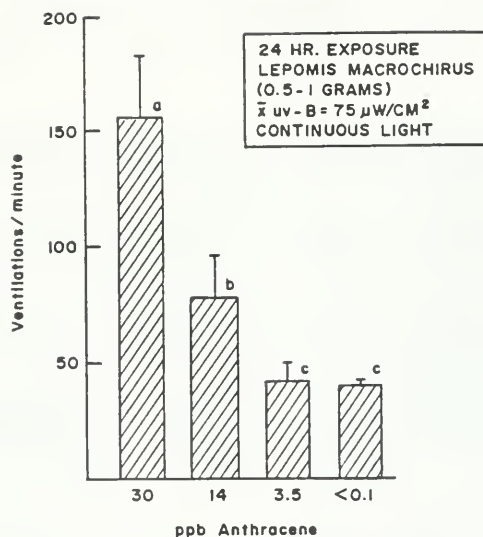


FIGURE 2.1.1. Opercular ventilation rate as a function of anthracene concentration in juvenile bluegill sunfish exposed concurrently to simulated sunlight. Error bars represent 2 SE, and $a > b > c$ (Student's T, $\alpha = 0.05$).

(From Oris 1985).

2.1.2.2 Subacute Effects

2.1.2.2.1 Teratogenic Effects in Fish

Studies on the teratogenic effects of PAH towards fish are limited to only a few compounds and are summarized in Table 2.1.5. The most intensive studies have been conducted by Hose *et al.* (1981, 1982, 1984) and Hannah *et al.* (1982), although they are limited to benzo[a]pyrene.

Hannah *et al.* (1982) and Hose *et al.* (1984) exposed rainbow trout eggs to various levels of B[a]P-contaminated sand which generated a range of aqueous concentrations up to 2.99 $\mu\text{g/L}$ (effectively a saturated solution). They observed a variety of terata (Table 2.1.5) which developed during organogenesis (day 7 to 24 post fertilization), although none appeared to be diagnostic of B[a]P or PAH in general. The maximum incidence of gross anomalies (14.3%) was almost five times the frequency observed in controls (2.6%) and the frequency in treated alevins was directly correlated with aqueous concentration ($r=0.89$, $p<0.001$). They also reported that embryonic tissue levels of B[a]P were 1.93 and 12.34 mg/kg wet wt. at 15 and 36 days post fertilization, respectively. This is a substantially lower tissue concentration than that employed by Black *et al.* (1985) in which embryonic B[a]P microinjections (136.1 mg/kg wet wt.) induced hepatic carcinomas in rainbow trout (see section 2.1.2.3.1). It suggests that enumeration of gross terata in trout alevins is useful as a rapid and sensitive evaluation of B[a]P impacts during early life stages.

The above study was also evaluated for terata at the histological level (Hose *et al.* 1984) which revealed certain anomalies were more frequent than gross anomalies indicated (e.g. microphthalmia, 17.1%). Skeletal deformities were associated with cartilage, vertebrae and the cranium. An important feature was that good correlation was observed between the incidence of terata and the frequency of erythrocyte micronuclei ($r=0.82$, $0.01<p<0.05$). This provides good evidence suggesting teratogenicity of this compound is mediated through a genotoxic mechanism.

TABLE 2.1.5
INFLUENCE OF TERATOGENIC EFFECTS OF PAH IN FISH

CHEMICAL	TEST ORGANISM	EXPOSURE ^a	TEMPERATURE °C	COMMENTS	REFERENCE
Acridine	Rainbow Trout <u>Salmo gairdneri</u>	0.410 mg/L (waterborne)	13.5	gross anomalies detected in 21% of exposed fish (no data provided on controls)	Black <u>et al.</u> 1983
	Largemouth bass <u>Micropterus salmoides</u>	11.50 mg/L (waterborne)	22.0	gross anomalies detected in 4% of exposed fish (no data provided on controls)	Black <u>et al.</u> 1983
Naphthalene	Rainbow trout <u>Salmo gairdneri</u>	0.230 mg/L (waterborne)	13.5	gross anomalies detected in 7% of exposed fish (no data provided on controls)	Black <u>et al.</u> 1983
	Largemouth Bass <u>Micropterus salmoides</u>	0.239 mg/L (waterborne)	22.0	gross anomalies detected in 6% of exposed fish vs 1% in fish exposed to 0.028 mg/l naphthalene	Black <u>et al.</u> 1983
Phenanthrene	Rainbow trout <u>Salmo gairdneri</u>	0.085 mg/L (waterborne)	13.5	gross anomalies detected in 43% of exposed fish (no anomalies detected at lower concentrations)	Black <u>et al.</u> 1983
Benzo(a)pyrene	Rainbow trout <u>Salmo gairdneri</u>	Sand(1-500 mg/Kg)	10	-frequency of gross anomalies: Hannah <u>et al.</u> 1982	
		+ Water(0.0008 to 0.003 mg/L)		control = 2.6% exposed = 5.3 to 14.3% -anomalies: kyphosis, immaturity, lack of retinal pigment, cyclopia, anophthalmia, microphthalmia, albinism, reduced yolk sac	

TABLE 2.1.5
INFLUENCE OF TERATOGENIC EFFECTS OF PAH IN FISH (Continued)

CHEMICAL	TEST ORGANISM	EXPOSURE ^a	TEMPERATURE °C	COMMENTS	REFERENCE
Benzo[a]pyrene (cont'd)	Rainbow trout <u>Salmo gairdneri</u>	Sand (1-500 mg/Kg) Water (0.00008 to 0.03 mg/L)	10.0	-microphthalmia most prevalent anomaly; 6.8 and 17.1% at 0.0002 and 0.0003 mg/L, respectively -skeletal deformities: cranial, vertebral, cartilage -anomalies correlated with erythrocyte micronuclei frequency ($r = 0.82$, $0.02 < p < 0.5$)	Hose et al. 1984
	English Sole <u>Parophrys vetulus</u>	Maternal transfer (maternal dose = 8 mg/kg body wt)	10.0	-frequency of gross anomalies: control = 1.6% exposed = 5.6% -anomalies: primarily deformities in yolk sac and/or tail region	Hose et al. 1981
	English Sole <u>Parophrys vetulus</u>	0.002 µg/L (waterborne)	11.5	-no significant difference in frequency of gross anomalies between control and exposed fish	Hose et al. 1982
	Flathead Sole <u>Hippoglossoides elassodon</u>	B[a]P + BSA 0.004 @ 0.002 mg/L respectively (waterborne)	11.0	no gross anomalies observed	Hose et al. 1982
	Sand Sole <u>Psetichthys melanostichus</u>	0.0001 mg/L (waterborne)	10.0	-frequency of gross anomalies: control = 0% exposed = 5% -anomalies: disorganized musculature, arrested development	Hose et al. 1982

Footnote: a - All exposures were initiated immediately following fertilization, unless otherwise specified

Teratogenic effects due to PAH exposure during early life stages may be highly relevant in terms of population survival since the effects are realized over a much shorter time span, and occur at a lower tissue concentration than apparently required in carcinogenicity studies. Moreover, PAH exposure at these concentrations and duration would, most likely, induce significant subtle impairments (e.g. behavioral, neurologic, reproductive). No efforts have been made in measuring these effects.

Additional studies by Hose et al. (1981) suggest B[a]P exposure of eggs, while still in the ovary (i.e. maternal transfer), may also give rise to teratogenesis in English sole (Parophrys vetulus), although histologically no toxic effect, neoplasia, precancerous lesions or anomalous mitotic figures could be detected. The frequency of terata (5.6%) compared to controls (1.6%) was marginally non-significant, however, eggs were allowed a maximum exposure time of only 5-h following a single oral maternal dose of 8 mg/kg. Hence, the results may be conservative in comparison to extended exposures mediated through feral fish indigenous to sediments highly contaminated with PAH.

Differences in frequency of terata were noted among three species of marine flatfish with sand sole (Psettichthys melanostichus) being the most sensitive (Table 2.1.5). Gross anomalies were again manifested during organogenesis (48-h post-fertilization) and the terata were similar to those observed in rainbow trout studies (e.g. disorganized somatic musculature and arrested development). In contrast to the marginal response observed for English sole larvae exposed via maternal transfer, no significant anomalies were observed when eggs were exposed to an aqueous concentration of 2.1 $\mu\text{g/L}$ B[a]P. Although not conclusive, this may suggest that maternal transfer of B[a]P is a more significant route of exposure.

Black et al. (1983) recorded the incidence of gross terata resulting from embryo-larval exposures of rainbow trout (Salmo gairdneri) and smallmouth bass (Micropterus salmoides) to acridine, naphthalene and

phenanthrene (Table 2.1.5). For trout, phenanthrene was the most potent teratogen, and is comparable to the effects evoked by B[a]P (Hannah et al. 1982). Bass were considerably less sensitive to acridine than trout; although exposed to three times the concentration used for trout, they exhibited only one fifth the incidence of gross anomalies. However, in the case of naphthalene, both species exhibited a comparable incidence of anomalies in response to similar aqueous exposures. No information was reported on the type of terata observed in this study.

2.1.2.2.2 Genotoxic Effects in Fish

Genotoxic effects include all impacts to DNA or chromosomal material in general, the more severe impacts being referred to as clastogenic effects. This may range from directly observable damage (e.g. chromosomal aberrations) to molecular interactions with DNA (e.g. covalent binding) which have the potential to mutate or break the molecule. Although there is often good correlation between genotoxicity and carcinogenicity, genotoxic effects are not evidence for carcinogenicity, but rather sentinels for its potential. Because of this correlation and the greater ease of testing for genotoxicity, many international agencies accept genotoxicity endpoints as evidence suggestive of carcinogenicity.

Micronucleus Assay

The micronucleus assay is a means to measure clastogenicity. It provides a semi-quantitative measure of fragmented chromosomal material arising from DNA breakage. Such fragments may become compartmentalized into smaller secondary nuclei and are conserved in daughter cells. This assay can be applied to any cell line, but it requires that a substantial fraction of the affected cell population undergo mitosis so that chromosomal fragments induced during the first

cell generation can be manifested as micronuclei in subsequent generations (Al-Sabti 1986). Although well utilized in mammalian studies, use of the micronucleus test in fish toxicology has received only limited attention. Two studies have addressed the genotoxicity of benzo[a]pyrene and 3-methylcholanthrene in three cyprinids and rainbow trout (Table 2.1.6).

Benzo[a]pyrene significantly increased the frequency of blood erythrocyte micronuclei in all species studied to date. This occurs regardless of route of entry (i.p. injection or direct aqueous uptake) or age (adult vs embryo-larval exposures). 20-methyl-cholanthrene was also effective in evoking a significant increase in micronuclei frequency in all three cyprinid species. The most notable features, however, are the dose response relationship and the comparable frequencies of micronuclei between two diverse groups of fish (ie. cyprinids vs salmonid): These features, along with its sensitivity and rapid rate of response suggest the assay would be an attractive screening test for comparing the relative genotoxic potential of PAH compounds in fish.

Hose et al. (1984) found a good correlation between erythrocyte micronuclei frequency in larval rainbow trout, exposed embryonically to benzo[a]pyrene and the observed rate of teratogenic effects in the same larvae. This suggests that the induction of at least some of the terata was likely a result of a genotoxic mechanism rather than a non-genetic mechanism.

Chromosomal Aberrations

Chromosomal aberrations quantified in mitotic or meiotic phases provide a direct measure of clastogenicity. Unlike the micronucleus test, this assay enables one to quantify direct damage to specific chromosomes and, in addition, characterize the damage. This provides the advantage of discerning between minor chromosomal anomalies (e.g. inclusion of fragments) and aberrations which are more likely to evoke cell death (e.g. chromosomal deletions).

TABLE 2.1.6
FREQUENCY OF MICRONUCLEI RESULTING FROM EXPOSURE TO
BENZO[a]PYRENE OR 3-METHYLCHOLANTHRENE

Chemical	Test Organism	Life Stage (g)	Exposure Concentration	Exposure Duration	Frequency of Cells with Micronuclei (#/1000 Cells)	Comment	Reference
Benzo[a]pyrene	Common Carp <u>Cyprinus carpio</u>	35-50	c	48 h	6.2	Significant dose-response relationship	Al-Sabti 1986
			10 $\mu\text{g/kg}$		21		
			40 (i.p.)		42		
			80		67		
	Tench <u>Tinca tinca</u>	35-50	c	48 h	6.5	Significant dose-response relationship	
			10 $\mu\text{g/kg}$		27		
			40 (i.p.)		34		
			80		71		
	Grass Carp <u>Ctenopharyngodon idella</u>	35-50	c	48 h	6.8	Significant dose-response relationship	
			10 $\mu\text{g/kg}$		22		
			40 (i.p.)		37		
			80		67		
	Rainbow Trout <u>Salmo gairdneri</u>	Embryo-Larval	c	36 d	2.9	Significant effect vs. control, but variable effect with dose	Hose et al., 1984
			0.08 $\mu\text{g/kg}$		9.2 ^a		
			0.21 (i.p.)		25.8		
			0.39		17.8		
			1.48		14.3 ^a		
			2.40		19.3		
			2.99		44.0		
3-Methylcholanthrene	Common Carp <u>Cyprinus carpio</u>	35-50	c	48 h	6.2	Significant dose-response relationship	Al-Sabti 1986
			10 $\mu\text{g/kg}$		30		
			40 (i.p.)		45		
			80		96		
	Tench <u>Tinca tinca</u>	35-50	c	48 h	6.5	Significant dose-response relationship	
			10 $\mu\text{g/kg}$		32		
			40 (i.p.)		37		
			80		36		
	Grass Carp <u>Ctenopharyngodon idella</u>	35-50	c	48 h	6.8	Significant dose-response relationship	
			10 $\mu\text{g/kg}$		19		
			40 (i.p.)		43		
			80		98		

Footnotes: a - Indicates not significantly different from controls, all other cases differ from control.

c - Control

Nichols et al. (1977) developed a classification for most anaphase aberrations as either 1) acentric fragments (fragments left at a equator during cell division); 2) attached fragments (fragments lagging behind chromosomes but apparently attached by a thread-like strand of chromatin); 3) chromosome bridges (units of chromatin stretched between two groups of anaphase chromosomes); 4) side arm bridges; and 5) tri or multipolar anaphase figures.

Research on fish chromosomal aberrations arising from PAH exposure is quite limited. Studies conducted in vitro on the rainbow trout gonad cell line (RTG-2) demonstrated a significant elevation in anaphase aberrations occurring within 24 to 48 h following exposure to 9-aminoacridine, benzo[a]pyrene, 3-methylcholanthrene, but not anthracene (Kocan et al. 1982). The data for benzo[a]pyrene also indicated a 10-fold increase in anaphase aberrations between 24 and 48 h such that over 90% of observed cells demonstrated aberrant chromosomes (Figure 2.1.2). This increase was attributed to a delay time required for RTG-2 cells to metabolize benzo[a]pyrene to reactive metabolites. This concept is supported by the fact that only a minor increase (less than 2-fold) in aberrations was observed for the direct acting mutagen 9-aminoacridine during the same period (Kocan et al. 1982). The lack of activity demonstrated by anthracene is consistent with its low carcinogenic activity toward laboratory animals (Eisler 1987).

The previously described aberration classification system (Nichols et al. 1977) was used to compare the action of the three mutagenic PAH (Table 2.1.7). After a 24 h-exposure, the aberration profile of the direct acting mutagen, 9-aminoacridine, was comparable to the promutagen, benzo[a]pyrene, except for a slightly higher frequency of bridges. However, the profile for benzo[a]pyrene may differ substantially following biotransformation to active diol epoxides. The profile of 3-methylcholanthrene was slightly more distinct, with a higher incidence of attached and acentric fragments, although this may only reflect a difference in exposure duration (48 h). In all three cases the primary effect was to increase the frequency of fragments and bridges only.

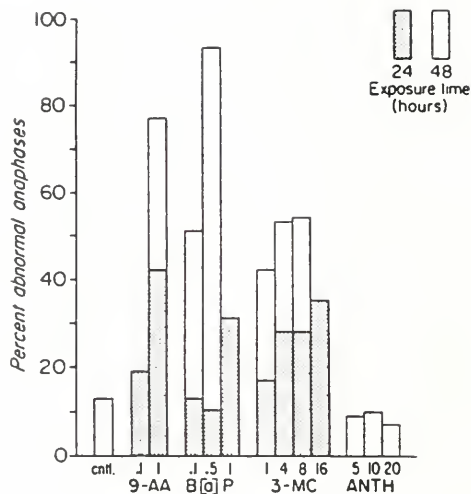


Fig. 2.1.2 Incidence of anaphase chromosomal aberrations in RTG-2 cells exposed to α -aminoacridine (9-AA), benzo[a]pyrene (B[a]P), 3-methylcholanthrene (3-MC) and anthracene (ANTH). Each bar represents the abnormalities observed in 200 anaphase figures, except that the control represent 1800 anaphase cells. Values on x-axis represent aqueous exposure concentration (mg/L).

(Kocan et al. 1982)

TABLE 2.1.7
TYPES OF ANAPHASE ABERRATIONS SEEN IN MUTAGEN-TREATED RTG-2 CELLS

Treatment	Number of cells scored	Attached Fragments (%)	Acentric Fragments (%)	Bridges (%)	Side-arm Bridges (%)	Multipolar Cells (%)
Control ^a	500	3.6	4.6	2.6	0.6	0.6
9-AA	200	18.5	9.0	13.5	0.5	1.0
B[a]P	200	13.5	8.5	7.5	0.5	1.0
3-MC	100	25.0	24.0	5.0	0	0

^a Control = untreated and DMSO-treated, 48 hr; 9-AA = 9-aminoacridine, 1 µg/ml, 24 h; B[a]P = benzo[a]pyrene, 1 µg/ml, 24 h; 1 µg/ml, 24 h; 3-MC = 3-methylcholanthrene, 4 µg/ml, 48 µr. (From Kocan *et al.* 1982)

Exposure to benzo[a]pyrene or 3-methylcholanthrene *in vitro* also resulted in chromosomal aberrations in three cyprinids (Table 2.1.8, Al-Sabti 1985). In this study, kidney cells were scored for aberrations 48 h following an i.p. injection of 10 to 80 mg PAH/kg body weight. Although frequency of aberrant cells generally increased with increasing dose, there was no significant dose-response relationship for a given species over the dose range studied. However, in all cases, treated fish had a significantly higher frequency of aberrant cells than sham injected controls. The range of frequencies is generally lower than that observed for cultured RTG-2 cells, but the predominant forms of aberrations are similar (e.g. attached and acentric fragments).

Non-adductive DNA Damage

Reactive metabolites of PAH (e.g. diol epoxides) may react with DNA in either an adductive (ie. covalent) or non-adductive manner (Shugart *et al.* 1987). In the latter case interaction with DNA is not covalent, but nevertheless may induce damage such as single strand breaks. Detection of this damage does not infer clastogenicity since DNA repair may subsequently occur. However, single strand DNA breakage can be regarded as a measure of potential for genetic damage i.e. clastogenicity, mutagenicity and carcinogenicity.

TABLE 2.1.8
MEAN FREQUENCY OF CHROMOSOMAL ABERRATIONS IN KIDNEY CELLS
OF CYPRINIDS 48 H FOLLOWING I.P. INJECTION OF
BENZO[a]PYRENE OR 3-METHYLCHOLANTHRENE

Chemical	Test Organism	Exposure (ng PAH/kg body wt)	No. of Fish Tested	Mean Frequency of Aberrant Cells % (n)
Benzo[a]pyrene	Common Carp <u>Cyprinus carpio</u>	Sham(H ₂ O)	6	4.2 (137)
		Sham(Corn Oil)	6	4.8 (151)
		10	5	15.8 (141)
		40	6	18.7 (133)
		80	4	23.1 (101)
	Tench <u>Tinca tinca</u>	Sham(H ₂ O)	6	3.5 (182)
		Sham(Corn Oil)	6	6.0 (131)
		10	4	17.9 (131)
		40	3	22.6 (112)
		80	3	24.0 (109)
	Grass Carp <u>Ctenopharyngodon idella</u>	Sham(H ₂ O)	6	4.8 (151)
		Sham(Corn Oil)	6	5.3 (142)
		10	5	16.5 (129)
		40	4	19.2 (115)
		80	4	20.4 (125)
3-Methylcholanthrene	Common Carp <u>Cyprinus carpio</u>	Sham(H ₂ O)	6	4.2 (137)
		Sham(Corn Oil)	6	4.8 (151)
		10	6	21.5 (105)
		40	6	21.3 (113)
		80	6	25.1 (98)
	Tench <u>Tinca tinca</u>	Sham(H ₂ O)	6	3.5 (182)
		Sham(Corn Oil)	6	6.0 (131)
		10	4	15.0 (143)
		40	4	18.0 (129)
		80	4	22.7 (123)
	Grass Carp <u>Ctenopharyngodon idella</u>	Sham(H ₂ O)	6	4.2 (151)
		Sham(Corn Oil)	6	5.3 (142)
		10	4	20.4 (119)
		40	4	12.5 (121)
		80	5	26.6 (108)

Shugart (1988) employed the alkaline unwinding assay to determine DNA damage in bluegill sunfish (Lepomis macrochirus) and fathead minnow (Pimephales promelas) following aqueous exposure to benzo[a]pyrene (1 $\mu\text{g/L}$). The assay is based on the principle that DNA strand separation occurs at single-strand breaks under defined conditions of pH and temperature. In the absence of single-strand breaks, double-stranded DNA (dsDNA) predominates following alkaline unwinding. However, increasing occurrence of single-strand breaks will yield a proportional increase of single-stranded DNA (ssDNA), with a concomitant decrease in dsDNA, provided DNA repair synthesis is prevented. Proportions of dsDNA and ssDNA are then determined fluorometrically (see Shugart 1988 for detailed technique). The assay is therefore a measure of clastogenic effects which excludes the ameliorating influence of DNA repair.

Figure 2.1.3 illustrates the progressive change in composition of hepatic DNA in exposed bluegill sunfish. This is expressed as the fraction (F) of total DNA which is present as dsDNA following unwinding. Over the first 16 days of exposure a rapid and significant decrease in dsDNA was observed. This was followed by a return to control levels over a similar time frame, and remained unchanged over the duration of the chronic exposure.

The results are intriguing since they suggest that an adaptive response occurred following the initial clastogenic insult. Shugart (1988) attributed the initial decrease in dsDNA to a rapid bioconcentration and biotransformation of B[a]P to its reactive and clastogenic metabolites. The decreased rate of decline observed after day 10, followed by a restoration of F to control levels may be related to an enhanced DNA repair ability, perhaps through enzymatic induction, although further studies are required to determine this.

The fraction of dsDNA in experimental (F_e) and control (F_o) samples was used to calculate the number of single-strand breaks (n) using the formula described by Kanter and Schwartz (1979):

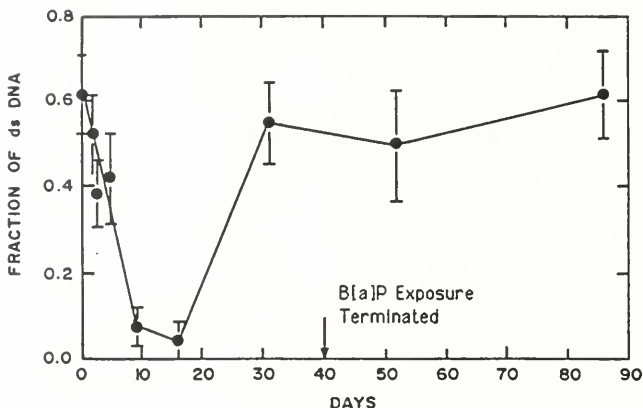


Fig. 2.1.3 Integrity of liver DNA of bluegill sunfish during chronic exposure to benzo[a]pyrene. Data expressed as fraction of double-stranded DNA present in fish examined. Each point is the average of data from four separate fish (+/- standard error of the mean).

(From Shugart 1988)

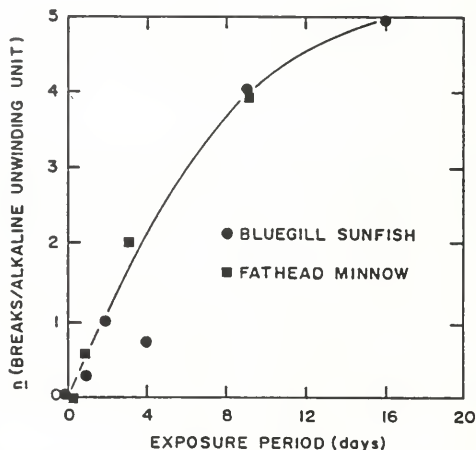


Fig. 2.1.4 Time course of benzo[a]pyrene-induced strand breakage in DNA isolated from bluegill sunfish and fathead minnows during initial phase of exposure. Number of strand breaks (n) determined using equation 2.1-1

(From Shugart 1988)

$$n = \frac{\ln F_e}{\ln F_0} - 1 \quad (\text{Equation 2.1-1})$$

When expressed as number of breaks per unit DNA over exposure time, the same trend was clearly evident for both species tested, suggesting a similar progression of clastogenic impacts (Figure 2.1.4). The results reflect a maximum rate in break formation during the initial exposure phase (approximately $0.75 \text{ breaks} \cdot \text{unit DNA}^{-1} \cdot \text{day}^{-1}$), followed by a gradual reduction towards zero (ie. plateau).

Similar clastogenic effects were observed in the mosquito fish (Gambusia affinis) following exposure to B[a]P at the same concentration as discussed above ($1 \mu\text{g/L}$, Batel et al. 1985). This study addressed the same clastogenic end point (ie. number of DNA breaks per unit DNA), but employed an entirely different technique. Nuclease S_1 treatment, electron microscopy and DNA mass and length determination were used to quantify and characterize the fragmented hepatic DNA following a 48 h in vivo exposure. While control fish exhibited a bimodal distribution in size of hepatic DNA, treated fish lacked the larger sized molecule (Figure 2.1.5). Thus the average size of DNA units in the treated fish was reduced to 2.6×10^7 daltons from a control value of 3.6×10^7 daltons. The authors calculated that B[a]P exposure introduced 0.31 to 0.46 breaks per control liver DNA molecule, a value comparable to that observed by Shugart (1988).

The two studies, therefore, demonstrate in vivo the capacity for B[a]P to induce clastogenic damage at concentrations attainable in field situations. They also demonstrate good agreement, and therefore validation, for the same endpoint measured through different approaches.

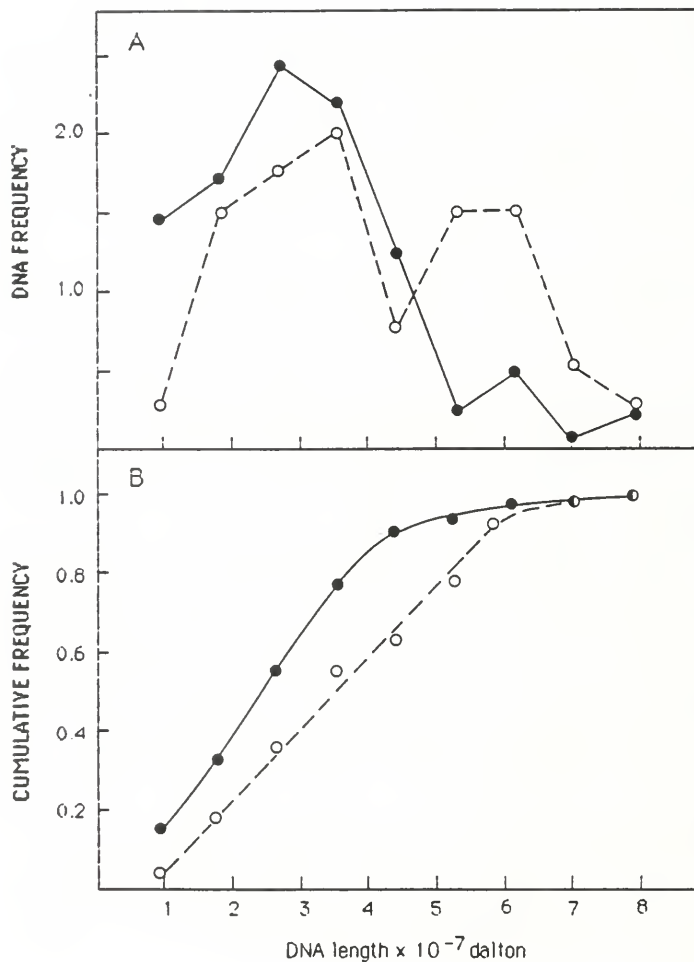


Fig. 2.1.5 Size distribution of nuclease S₁ treated double-stranded DNA from female fish liver: (○) controls; (●) exposed to water containing 100 ppb benzo(a)pyrene for 2 days. (A) The percentage of molecules per class and (B) the cumulative frequency distribution are shown versus the molecular weight classes, expressed in Daltons.

(From Shugart 1988)

DNA Adduct Formation

A widely held theory is that PAH-induced carcinogenesis is at least partially derived through the covalent adduction of electrophilic intermediates to DNA (Heidelberger 1976, Miller and Miller 1981). In support of this theory is the dose-response relationship between the formation of the 7R anti-BPDE/trans dG adduct in mice and the ensuing skin papillomas which are observed (Ashurst and Collier 1980). Moreover, the rate of excision repair of this adduct in mouse skin is comparatively slower than that observed in rats, a species which is relatively more resistant to B[a]P-induced carcinogenesis. Thus data concerning the formation of DNA adducts with PAH intermediates is an important means of gaining insight towards the carcinogenic risk of PAH-exposed organisms.

Research in this area conducted on fish has been approached through in vitro and in vivo techniques. The in vitro studies have largely been conducted by Ahokas, Varanasi and co-workers. Species examined include the English sole, starry flounder, coho salmon, lake trout and roach. It appears that the research to date has exclusively addressed the adduction of B[a]P intermediates only. Essentially the research involves the isolation of hepatic microsomal system from either uninduced (control) or induced specimens and incubating this fraction with salmon sperm DNA and radio-labeled B[a]P. Isolation of the DNA adducts on Sephadex columns then enables derivation of the B[a]P/DNA binding activity (Table 2.1.9).

The studies conducted by Varanasi and co-workers clearly show an increase in DNA binding activity in response to previous exposure of the intact organism to isolated PAH (e.g. 3-MC and B[a]P) and mixtures (e.g. Prudhoe Bay Crude Oil). The enhanced binding as a result of pre-exposure ranges from 500 to 5300% over controls. While one should bear in mind that many other factors beyond DNA binding are involved in determining carcinogenic risk and susceptibility (e.g. age, excision repair), coho salmon demonstrated the largest increase over controls in binding capacity as a result of pre-exposure. However, on the basis

TABLE 2.1.9
COVALENT BINDING OF BENZO[a]PYRENE TO DNA
TO FISH SPERM CONDUCTED IN VITRO

Species	Pre-Exposure ^a (PAR\Dose\Duration)	Hepatic Microsomal Activating System	DNA Source	Incubation Time\Temp. (min/°C)	B[a]P Binding fmole equivalents/ mg DNA/mg Protein	% Control Value	Reference
Starry Flounder	Control	5 pmole B[a]P 5 mg Protein	2 mg Salmon Sperm	15/25	150	100	Varanasi and Gaur, 1980.
" "	MC/10mg Kg ⁻¹ /24hr	"	"	"	1620	1100	"
" "	B[a]P/10mg Kg ⁻¹ /24hr	"	"	"	1700	1100	"
" "	PBCO/10mg Kg ⁻¹ /24hr	"	"	"	740	500	"
" "	MC ^b /10mg Kg ⁻¹ /24hr	"	"	"	530	NA	"
English Sole	Control	"	"	"	60	100	Varanasi et al., 1980
" "	PBCO/10 mg Kg ⁻¹ /24hr	"	"	"	1050	1800	"
" "	MC ^b /10mg Kg ⁻¹ /24hr	"	"	"	160	NA	"
Coho Salmon	Control	"	"	"	20	100	Varanasi and Gaur, 1980
" "	MC/10mg Kg ⁻¹ /24hr	"	"	"	970	4900	"
" "	B[a]P/10mg Kg ⁻¹ /24hr	"	"	"	1060	5300	"
" "	MC ^b /10mg Kg ⁻¹ /24hr	"	"	"	300	NA	"
Rat	Control	"	"	15/37	60	100	"
"	MC/10mg/Kg ⁻¹ /24hr	"	"	"	690	1200	"

TABLE 2.1.9 (continued)
COBINDING OF BENZO[a]PYRENE TO DNA
TO FISH SPERM CONDUCTED IN VITRO

Species	Pre-Exposure ^a (PAH\Dose\Duration)	Hepatic Microsomal Activating System	DNA Source	Incubation Time\Temp. (min/°C)	B[a]P Binding fmole equivalents/ mg DNA/mg Protein	% Control Value	Reference
Lake Trout	None ^b	60 nmole B[a]P 4 mg Protein	20 mg Salmon	30/37	170	319	Ahokas et al., 1979
Roach	None ^b	"	"	"	5	11.2	"
Rat	Control	"	"	"	55	100	"
"	MC/3x25mg Kg ⁻¹ /72hr	"	"	"	1950	3634	"

Footnotes: a - All pre-exposure were mediated through i.p. injections using corn oil or vegetable oil.

b - All-fish pre-exposures were conducted at 13°C except where noted g(9) 8°C; (bb) 4-6°C.

MC - 3-methylcholanthrene

B[a]P - Benzo[a]pyrene

PCBO - Prudhoe Bay Crude Oil.

of maximum induced in vitro binding activity, all three fish species had comparable values. In contrast, the efficacy of induced Sprague-Dawley rat liver microsomes to form DNA adducts in vitro was notably lower, and this may reflect the general resistance of this species to B[a]P induced hepatocarcinogenesis (Langenbach et al. 1983).

Studies conducted by Ahokas et al. (1979) on uninduced lake trout and roach demonstrated B[a]P-DNA binding activities comparable to control fish in studies conducted by Varanasi and co-workers. In addition, the former study determined MFO activity of the hepatic microsomes employed for binding experiments, the results closely reflected binding activity, with roach clearly having the lowest values for MFO activity.

To further elucidate the significance of in vitro binding of B[a]P metabolites with DNA, studies were conducted to specifically identify the B[a]P mutabilities involved in the adduction of DNA. Using tritiated B[a]P and the racemic 7,8-dihydrodiols, Nishimoto and Varanasi (1985) demonstrated that English sole hepatic microsomes, in the presence of salmon testes DNA formed a single major adduct (60-68% of the total modified deoxynucleotides). These findings indicated that English sole microsomes in vitro stereo-selectively metabolize B[a]P to the mammalian carcinogen anti-BPDE. The results support the observed propensity of neoplasia in feral English sole collected from areas highly polluted with PAH in Puget Sound.

In contrast to English sole liver microsomes, Ahokas et al. (1979) found that microsomes of lake trout and 3-MC-induced rats produced at least two major DNA adducts. Using Sephadex chromatography, one peak was identified as the adducts of 7,8-BPDE, consistent with that observed in English sole. In addition, a second and somewhat larger peak was identified as the adduct of 9-OH-BP-4,5-oxide.

The above studies illustrate that hepatic microsomes from a variety of

fish species have ability to form electrophilic agents from bay-region containing PAH compounds, in turn leading to formation of covalently bonded DNA adducts. Further, the metabolites and resulting adducts formed by fish are identical to those leading to carcinogenicity in mammals. These studies may not accurately describe the situation in vivo since in vitro binding of B[a]P derived moieties to DNA is not strictly correlated to mutagenicity or carcinogenicity. These studies generally support the idea of using fish species as surrogates for carcinogen testing or as sentinel animals to detect PAH carcinogens in the environment, however, it is likely that different fish species probably vary in their relative abilities to generate, detoxify, and excrete PAH-derived metabolites.

Accordingly, in vivo studies on DNA adduct formation have been performed to more thoroughly resolve the carcinogenic potential of PAH towards fish. Two approaches are currently being employed in this area; the first is an extension on the in vitro studies in which radio-labeled B[a]P (^3H or ^{14}C) is provided as an in vivo exposure and DNA adducts are then isolated from liver cells (Varanasi et al. 1981b, 1983, 1986 and von Hofe and Puffer 1986). A second approach which is currently attracting much attention is the ^{32}P post-labeling technique of in vivo derived DNA adducts (Dunn et al. 1987, Maccubin, et al. 1987b, Shugart et al. 1987).

In vivo DNA adduct formation in fish as a result of radio-labeled B[a]P exposure has been reported for a variety of fish species including sanddab, California killifish, English sole and starry flounder and compared to that found for Sprague-Dawley rats and mice (Strain C57-B1/6J); (Varanasi et al. 1981b, 1982a, 1983, 1986 and von Hofe and Puffer 1986). The data has been summarized in Table 2.1.10 and is expressed somewhat differently than the in vitro data discussed above. The covalent binding activity is expressed on the basis of fmole B[a]P equivalents/mg DNA. In order to facilitate between study comparisons Varanasi et al. (1981b) implemented the Covalent Binding Index (CBI) which expresses the binding activity as a

function of the theoretical dose administered to the organism. From Table 2.1.10 it is apparent that B[a]P binding activity may vary substantially both within and among species. The greatest influence on binding activity appears to be mediated through the combination of i.p. injection with an acetone vehicle (e.g. English sole, starry flounder). This exposure regime presumably enhances entry of the PAH to the site of metabolism, while the more non-polar oils would likely not facilitate desorption of B[a]P from the vehicle to the same extent.

Comparisons within and between studies are nevertheless possible if such modifying effects are borne in mind. In the case of B[a]P exposure via acetone i.p. injection, English sole and starry flounder exhibited binding activities and CBIs 50 to 90 times greater than that observed for the Sprague-Dawley rat, a species that is generally resistant to B[a]P-induced liver neoplasia (Langenbach et al. 1983). The difference between fish species is also consistent with a greater frequency of neoplasia observed in feral English sole versus starry flounder from Puget Sound. Both the B[a]P binding activity and the CBI recorded for English sole, exposed per os to 0.1 or 2.0 mg B[a]P/kg body weight via corn oil, suggest a direct dose-response relationship exists. For a 20-fold increase in dose, the B[a]P binding activity increased 40-fold while the CBI increased 20-fold.

Studies conducted by von Hofe and Puffer (1986) resulted in CBIs for speckled sanddab and California Killifish consistently lower than those of the Pleuronectids investigated by Varanasi and co-workers, despite remarkably similar B[a]P metabolite profiles. B[a]P binding in these fish was also five to eight times lower than that observed in mice given equimolar injections. This result is notable in view of the fact that both fish species generate B[a]P-7,8-diol, a precursor of the reactive anti-BPDE which is most conducive to covalent binding (von Hofe and Puffer 1986). Mice, however, are known to yield a high proportion of the B[a]P-4,5-diol, the precursor of a less reactive B[a]P-diol epoxide (Holder et al. 1985). These observations suggest

an additional factor such as DNA repair may be influencing the CBIs of these fish.

Varanasi et al. (1987) note that differences in metabolic activation, as discerned from microsomal metabolite profiles in fish and rodents alone cannot explain the observed differences in covalent binding of B[a]P to hepatic DNA in vivo. A factor which may be implicated in explaining these differences is likely in vivo detoxification processes. Evidence which suggests this phenomenon lies in the metabolite profile of these species. For example, among the four fish species shown in Table 2.1.10, the metabolite profiles indicate B[a]P-7,8-diol constitutes 20-30% of the total metabolites (Varanasi et al. 1986b, von Hofe and Puffer 1986). However, the observed range of CBIs among these species is substantially larger, even after one makes allowances for the influence of exposure route and vehicle. Undoubtedly, the integration of detoxification processes such as different rates of phase II reactions and rates of excision repair may help to resolve some of the observed peculiarities.

In vivo covalent binding of B[a]P to DNA has been used to indirectly demonstrate the efficacy of excision repair in English sole hepatic DNA. Figure 2.1.6 profiles the level of DNA adducts (CBI) as a function of time following a single i.p. or p.o. exposure to ³H-B[a]P. The CBI for i.p. exposure indicates a rapid increase in DNA adducts within the first 8 to 48 h following administration of the dose. This level of DNA modification, however, fails to demonstrate a significant reduction over as much as 30 days, suggesting that excision repair in this species is relatively poor. This would also support the high prevalence of hepatocarcinogenesis in this species.

Additional in vivo B[a]P-DNA binding studies have revealed that juvenile English sole demonstrate a significantly higher CBI than that observed in adult sole (Table 2.1.10, Figure 2.1.7). This occurs despite the fact that neoplasms are more prevalent in adults (Varanasi et al. 1987). Hence, these observations suggest that the higher incidence of hepatocarcinogenesis in adult sole reflects the length of

time after initiation required for neoplasia to be manifested as overt tumors, rather than differences in susceptibility as a function of age.

Limited work has addressed the influence of gender on covalent binding of B[a]P to DNA. No differences were observed in the hepatic CBI between male and female English sole (Varanasi et al., 1982a). Interestingly, the data collected from this study which involved a per os dose of approximately 10 μ g B[a]P/kg body wt demonstrated considerable variability in CBI over 168 h, similar to that observed for the oral dose in Figure 2.1.6. In the same study it was also noted that the level of DNA adducts formed in the testes of English sole after 168 h was comparable to that present in hepatic DNA, with CBI's of 1.5 and 2.8, respectively. This observation suggests there is a significant potential for teratogenic effects to be mediated through modified DNA of gametes.

An alternative and relatively novel technique being employed in the detection of DNA adduct formation in fish and invertebrates involves the ^{32}P postlabeling of aromatic DNA adducts in liver tissue (Shugart et al. 1987, Stein et al. 1987, Dunn et al. 1987, Kurelec et al. in press). Recently, the development and further refinement of this technique suggests extremely sensitive detection of specific classes of DNA adducts are possible, among those being PAH metabolites (Randernath et al. 1981, Gupta et al. 1982, Reddy et al. 1984, Reddy and Randernath 1986). A promising advantage to this technique over those previously described is that it does not require the xenobiotic to be radio-labeled in order for detection, identification and quantification to be possible. It thus lends itself to studies which integrate both environmental (field) and laboratory exposures for the evaluation of ecosystem health.

TABLE 2.1.10
COVALENT BINDING OF BENZO(a)PYRENE TO
HEPATIC DNA CONDUCTED IN VIVO

Species ^a	Dose (μ g B[a]P/ kg Body weight)	Vehicle	Route of Exposure ^c (Duration hr)	B[a]P Binding Activity (fmol equivalents/ μ g DNA)	% Admin. Dose in liver	CBI ^b	Reference
California Killifish	0.6	Corn Oil	i.p. (24)	8.9	NA	1.2	von Hofe & Puffer, 1986
Speckled Sanddab	0.6	Corn Oil	i.p. (24)	14.0	NA	1.8	von Hofe & Puffer, 1986
English Sole	0.1	Corn Oil	p.o. (24)	51.0	0.8	45.0	Varanasi <i>et al.</i> , 1981
	2.0	Corn Oil	p.o. (24)	2100.0	0.43	85.0	Varanasi <i>et al.</i> , 1986
	2.0	Corn Oil	i.p. (24)	250.0	0.78	9.8	Nishimoto & Varanasi (unpub)
	2.0	Acetone	i.p. (24)	28000.0	6.0	1090.0	Varanasi <i>et al.</i> , 1986
English Sole (≤ 3 years)	0.1	Corn Oil	p.o. (24)	40.0	0.8	31.0	Varanasi <i>et al.</i> , 1983
English Sole (≥ 6 years)	0.1	Corn Oil	p.o. (24)	5.0	0.4	3.9	Varanasi <i>et al.</i> , 1983
Starry Flounder	2.0	Corn Oil	p.o. (24)	540.0	0.6	21.0	Varanasi <i>et al.</i> , 1986
	2.0	Acetone	i.p. (24)	14000.0	3.5	545.0	Varanasi <i>et al.</i> , 1986
Rat (Sprague-Dawley)	2.0	Acetone	i.p. (24)	300.0	1.0	12.0	Varanasi <i>et al.</i> , 1986
Mouse (C57Bl/6J)	0.6	Corn Oil	i.p. (24)	72.0	NA	9.3	von Hofe & Puffer, 1986

Footnotes: a - All values are for both sexes combined, except for rat and mouse, where only females and only males were used, respectively.

b - Covalent Binding Index (CBI) = $\frac{\mu\text{mole B[a]P equivalents}}{\mu\text{mole nucleotides}}$, where Mwt B[a]P = 252.3 g and 1 mole is taken $\mu\text{mole B[a]P administered/kg body wt.}$

to be 309 g.

c - i.p. - Intraperitoneal Injection, p.o. - per os

d - Exposures were conducted at 20°C, all other fish exposures were conducted at 12°C.

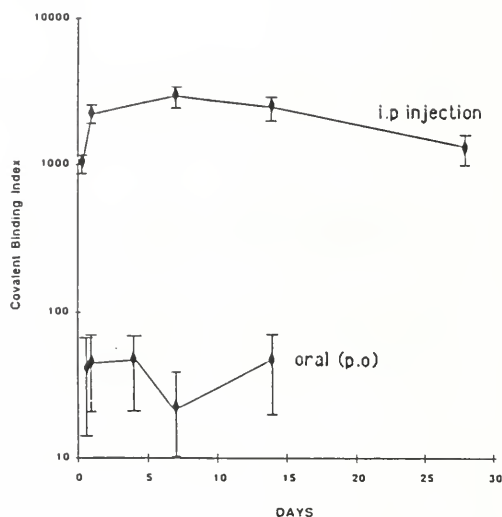


Fig. 2.1.6 Persistence of B[a]P-DNA adducts in liver of juvenile English sole exposed to ^3H -B[a]P via i.p. injection [(2 mg B[a]P dissolved in acetone)/kg body weight] or p.o. [(0.1 mg B[a]P, dissolved in corn oil)/kg body weight].

(From Varanasi et al. 1987)

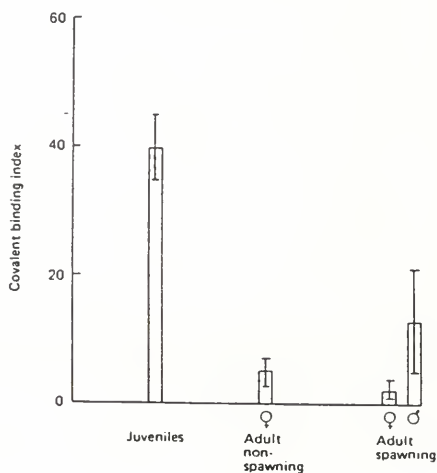


Fig. 2.1.7 Covalent binding induces of benzo[a]pyrene metabolites to hepatic DNA of juvenile and adult English sole exposed p.o. to 0.1 mg ^3H -B[a]P/kg body weight. Hepatic DNA was isolated from fish 24 hr. following dose. The covalent binding index (CBI) = ($\mu\text{mole B[a]P}$ -equivalents/mole nucleotide)/(mmole B[a]P administered/kg body weight).

(From Varanasi et al. 1987)

However, because analysis of DNA adducts using the ^{32}P post-labeling technique is still in the developmental phase, its use to interpret environmental impacts at this stage is somewhat unsure. For example, it is not yet clear whether an observed elevated frequency of hepatic DNA adducts truly represents a deleterious effect, since DNA repair efficiency may vary from species to species. Although there is a weight of evidence suggesting that adducts can cause mutational events leading to cancer, a causal relationship is not confirmed.

The principle of the ^{32}P post-labeling technique involves isolation, purification and hydrolysis of hepatic DNA from exposed fish (laboratory or feral specimens). The sensitivity of the assay is partially facilitated through enrichment of hydrophobic adducts using reverse phase high performance liquid chromatography and nuclease P1 enrichment for bulky adducts. These samples are then labeled using [γ - ^{32}P]-ATP and polynucleotide kinase. Samples are spotted on thin layer chromatography (TLC) plate for selective detection of aromatic nucleotide-carcinogen adducts by autoradiography. Quantification of ^{32}P adducts is accomplished through scintillation counting of isolated TLC spots.

Shugart et al. (1987) employed the ^{32}P post-labeling technique under controlled laboratory exposures of bluegill sunfish (Lepomis macrochirus) to B[a]P through either acute i.p. injection or chronic aqueous uptake. DNA adducts of both the anti- and syn- isomeric forms of BPDE were detected within 24 h of exposure. Particularly important was that anti-BPDE, a potent mammalian carcinogen, accounted for approximately 85% of the adducts formed which is very similar to the situation observed in English sole (Nishimoto and Varanasi 1986). This suggests bluegill sunfish may also be at high risk of hepatocarcinogenesis in the presence of B[a]P and possibly a variety of other PAH. The level of adduct formation was similar to that reported for mice and was dependent upon acclimation temperature (see Modifying Factors, section 2.4.1) and previous exposure to 3-methylcholanthrene.

Further studies conducted by Shugart et al. (1987) suggest bluegill sunfish, like English sole (Varanasi et al. 1987) have a poor ability to repair DNA damage, as adducts arising from 30-day aqueous exposure of only 1 µg/L B[a]P (a realistic environmental concentration) persisted for 45 days following the termination of exposure.

The ^{32}P post-labeling technique has also been employed in the examination of feral fish from the Buffalo and Detroit rivers, areas known to contain high levels of PAH contamination and demonstrate a high incidence of fish neoplasia (Black 1983a,b, Fallon and Horvath 1985, Dunn et al. 1987). Autoradiograms of chromatographs of brown bullhead catfish (Ictalurus nebulosus) from these sites demonstrated a diffuse diagonal zone corresponding to DNA adducts which was not evident in chromatographs from control bullheads. Total DNA adduct frequency from Buffalo River fish (70.1 ± 29 nmole adduct/mole normal nucleotide, $n=21$) and Detroit River fish (54 nmole adduct/mole normal nucleotide, $n=2$) were notably elevated in comparison to laboratory-reared controls (12.8 ± 9.2 nmole adduct/mole normal nucleotide, $n=6$).

Although it could not be established if the observed adducts were indeed derived from the carcinogens known to be present at the field sites, the characteristics of the adducts indicated they were of the pollution-related aromatic hydrocarbons.

A final means of assessing DNA adduct damage involves the acid-induced removal of diol epoxides adducts from macromolecules as the strongly fluorescent free tetrols (Shugart et al. 1987). Subsequently these are identified and quantified by HPLC and fluorescent spectroscopy. In the case of B[a]P, tetrols arising from the anti- and sys- diol epoxides were distinguished and quantified in bluegill sunfish following a dose of 5 mg B[a]P/kg body wet weight (Table 2.1.11).

Particularly noteworthy is the predominance (85%) of tetrols derived from anti-B[a]P diol epoxide, the most carcinogenic metabolite of B[a]P. The data also indicate that covalent binding with hemoglobin

and tetrol derivatization correlate well with DNA adduction. In vivo adduction to both macromolecules is enhanced at warmer temperatures, presumably due to more rapid formation of metabolites through the MFO system. However, the temperature does not influence the relative abundance of each metabolite.

The frequency of adduct formation in fish is comparable to that observed in the mouse using the same analytical technique, suggesting impacts on the two model organisms are mediated through comparable mechanisms.

2.1.2.2.3 Biochemical and Physiological Effects

Within animal tissues, PAH cause toxicity by binding to lipophilic sites, particularly on the outside of cell membranes (Neff, 1979). Binding of PAH molecules causes a perturbation of membrane surface organization and this, in turn, may disrupt essential cell processes such as ion exchange, or disturb the behavior of membrane-bound enzymes. This disturbance at the cellular level may translate into physiological disturbance of osmotic balance, muscle contraction or neurotransmission (DiMichele and Taylor 1978, Neff 1979). While the heavier PAH with very high lipid solubility, are more prone to accumulate in animal tissue, and therefore likely to evoke these cytological disruptions, lighter PAH (e.g. naphthalene) with greater solubility may be more readily available for uptake (DiMichele and Taylor 1978).

Toxicity of PAH mixtures has been assessed primarily in relation to petroleum mixtures, since PAH contribute to the water-soluble portion of crude or refined oils (Anderson et al. 1974). These studies have used either whole crude, refined oils, or the water soluble fraction (e.g. Woodward et al. 1987) or artificial mixtures designed to simulate the composition of toxic compounds in natural oil (Palawski et al. 1985, Hall et al. 1984). Unfortunately, PAH may constitute only a small percentage of the water-soluble fraction of oil (as low

TABLE 2.1.11
ADDUCT FORMATION IN THE MOUSE AND BLUEGILL
SUNFISH FOLLOWING A 72-h ACUTE EXPOSURE TO
BENZO[a]PYRENE

Test Organic	Macro Molecule	Exposure (mg/kg/wet/wt)	Adduct Formation ^c		
			Tetrol I-1	Tetrol II-2	% I-1
Bluegill ^a Sunfish	Hepatic DNA	5	928	127	88
	Hemoglobin	(i.p.)	13.8	4.6	75
Bluegill ^b Sunfish	Hepatic DNA	5	340	62	85
	Hemoglobin	(i.p.)	2.9	0.9	76
Mouse	Skin DNA	3 (topical)	588	220	73
	Hemoglobin	10 (topical)	2.9	nd	100

Footnotes: a - Fish were maintained at 20 °C during exposure
b - Fish were maintained at 13 °C during exposure
c - Concentration of tetrols (ie. adducts) expressed as
nanograms of tetrol per gram of macromolecule

(Modified from Shugart et al. 1987)

as 1% in some oils) and many other compounds present are also highly toxic (e.g. phenols). Hence, petroleum toxicity data may be of limited value in assessing PAH toxicity.

Wong and Englehardt (1982) exposed marine and freshwater acclimated rainbow trout to either benz[a]anthracene or crude oil for 7 days and assessed the effects on various gill ATPases. While freshwater marine acclimated fish were generally more sensitive to the petroleum mixtures than those acclimated to marine conditions, benz[a]anthracene evoked little response from either group of fish, with the exception of Na-K⁺ ATPase (Table 2.1.12). Where significant effects occurred, they usually involved inhibition of the ATPase. Impairment of gill ATPases provides a biochemical basis for osmotic and ionic imbalances observed in similar studies with rainbow trout (Englehardt et al. 1981b). In this study, freshwater acclimated trout experienced a significant decrease in plasma Cl⁻, Na⁺⁺ and K⁺, but no change in Ca⁺⁺. Marine acclimated trout exhibited the opposite response, in which plasma ions increased suggesting that ionoregulation by these ATPases was compromised.

In addition to the gill biochemical lesions noted above, hydromineral balances will also be influenced through changes in plasma cortisol concentrations. This general response to a stressor evokes a variety of physiological changes including alterations to the hydromineral balance as a result of increased gill permeability (Pickering 1981). In the present study, plasma cortisol levels in trout were significantly increased in treated fish. Naphthalene, one of the more common PAH found in the water soluble fraction of crude oils, also evoked increased serum cortisol glucose and protein in the mummichog Fundulus heteroclitus (DiMichele and Taylor 1978).

Substantial subacute effects were observed in spot (Leiostomus xanthurus) within 8 days of exposure to sediments highly contaminated with PAH originating from the Elizabeth River, Virginia, U.S.A (Hargis et al. 1984). Sediment PAH content ranged from 2500 to 3900 mg/kg dry wt. Concentrations of the twenty most concentrated compounds are listed in Table 2.1.13.

TABLE 2.1.12
SUBACUTE EFFECTS OF SELECTED
PAHs ON BIOCHEMICAL PARAMETERS OF FISH

Chemical	Test Organisms	Concentration (mg/L)	Exposure Duration (days)	Effect	Reference
Naphthalene	Mummichog	0.002-0.002 ^a	15	- significant increase in serum cortisol	DiMichele & Taylor 1978
	<u>Fundulus heteroclitus</u>	0.002-0.02	15	- significant increase in serum glucose	
		Control-0.02	15	- significant increase in serum protein	
Benz[a]anthracene	Rainbow Trout <u>Salmo gairdneri</u> (freshwater)	0.03	7	- no significant effect on gill ATPases (Na^+/K^+ , $\text{Na}^+/\text{NH}_4^+$, HCO_3^- , Mg^{++} or Ca^{++})	Wong & Englenhardt 1982
	Rainbow Trout <u>Salmo gairdneri</u>	0.03	7	- significant reduction in Na^+/K^+ ATPase, but not $\text{Na}^+/\text{NH}_4^+$, HCO_3^- , Mg^{++} or Ca^{++})	

Footnote: a - Range given is the No Observable Effect Concentration (NOEC) and the Lowest Observable Effect Concentration (LOEC), respectively.

TABLE 2.1.13
CONCENTRATIONS OF SELECTED PAH OBSERVED IN
SEDIMENT FROM THE ELIZABETH RV., VA, U.S.A.
(conc. in mg/kg dry sediment)

Chemical	Range	Chemical	Range
Benzothiophene	nd	Benzo[a]fluorene	65-86
2-Methylnaphthalene	15-20	Benzo[b]fluorene	63-82
1-Methylnaphthalene	25-47	Benzo[a]anthracene	60-82
Biphenyl	8-16	Chrysene	78-105
Fluorene	75-137	Benzo[fluoranthrene	73-94
Dibenzothiophene	23-51	Benzo[e]pyrene	33-35
Phenanthrene	268-468	Benzo[a]pyrene	35-43
Anthracene	85-125	Perylene	10-12
Fluoranthracene	230-324	Indeno[1,2,3-cd]pyrene	13-20
Pyrene	155-226	Benzo[ghi]perylene	13-16

(Data from Hargis et al. 1984)

Mortalities and gross skin lesions were observed after 8 days of exposure, by 18 days mortalities amounted to 30%. Fish exposed only to the overflow water from a tank containing sediment exhibited better survival (>92 %), although skin lesions persisted. Additional subacute effects included anemia, opacity of the eyes, severely eroded fins and cessation of growth. The concentration of PAH in the sediment was exceptionally high, and it should be noted that spot are bottom feeders which would maximize exposure.

A very important feature of this study is that exposure tanks were located outside and subject to ambient variation (Hargis et al. 1984). It is therefore highly likely that photoinduced toxicity was a factor in this study (see section 2.4 for a discussion of photoinduced toxicity). This is supported by the similarity between the lesions observed in the present study and those described by Oris and Giesy (1985) for bluegill sunfish exposed to anthracene and ultra-violet light. Since histological examination of the skin lesions failed to demonstrate a bacterial etiology, this further support the hypothesis

that tissue damage was mediated through free radicals derived from photo-activation of endogenous PAH. Many of the compounds noted in Table 2.1.13 are known to be photo-activated in the presence of ultra-violet radiation (see Table 2.4.1 for examples).

Studies conducted in the field and laboratory have noted liver enlargement following prolonged exposure to PAH. Metabolic responses of fish to contaminant exposure often cause tissue enlargement which can be assessed as the tissue somatic index (TSI; ratio of organ wt to total body wt multiplied by 100). However, TSIs have no specific diagnostic value since they may vary due to other factors such as starvation or high dietary carbohydrate consumption (NRCC 1985). Nevertheless, the liver somatic index (LSI) can be useful as a simple indication of pathology, enhanced metabolic activity, or hepatic response to xenobiotic challenge.

Fabacher and Bauman (1985) reported enlarged livers in brown bullhead catfish (Ictalurus nebulosus) from rivers having sediments highly contaminated with PAH and other organics. Fish collected from control sites had a typical liver somatic index (LSI) of 1.85 to 2.01, while those from contaminated sites ranged from 3.4 to 3.63. It was also noted that no differences in specific activity of MFO existed between the two groups, suggesting, the LSI was a more sensitive indication of stress for this species.

Melius and Elam (1983) also noted significant enlargement of the liver in the sea catfish (Arius felis) following i.p. injections of 20-methyl- cholanthrene over a period of 7 days. This was also accompanied by significant increase in microsomal protein per unit liver mass. This evidence along with data on MFO activity indicated injected fish not only had a greater microsomal MFO capacity per unit weight of liver, but also more liver per unit body weight. The liver enlargement, therefore, appears to be a metabolic response to address the toxicant challenge.

2.1.2.2.4 Mixed Function Oxidase Activity

The mixed function oxidases (MFO) are a well known and important group of inducible enzymes primarily located in hepatic microsomes and assume a major role in the biotransformation PAH to nontoxic complexes and carcinogenic intermediates. As the name implies the net effect of these enzymes is to oxidize certain compounds, rendering them more polar or hydrophilic, which facilitates their ultimate excretion after conjugation. While the MFO system is intimately associated with corticosteroid hormone metabolism in mammals and fish, an additional role involves metabolism of a variety of xenobiotics of which PAH have been classically associated. Detailed accounts of the MFO system in fish and its comparison to mammalian MFOs have been reviewed by Lech et al. (1982) and Lech and Vodcnik (1984). The intent of the following discussion is to briefly review the association of MFO activity in freshwater fish in relation to PAH exposure.

Numerous earlier studies addressed the role of MFO system as a potential indicator of recent exposure to petroleum hydrocarbons, since such exposures demonstrated inducible microsomal activity above basal levels (Payne 1976, 1977, Payne and May 1979, Payne and Fancey 1982, Payne et al. 1984, 1985, Payne and Penrose 1975).

This view was also extended, or perhaps refined, to PAH exposure owing to their presence in petroleum hydrocarbons and ability to function as a substrate for certain MFO enzymes (e.g. benzo[a]pyrene hydroxylase). It has, however, become increasingly apparent that the MFO system cannot be used as a diagnostic assay for feral PAH exposure since numerous other contaminants also induce this system (e.g. TCDD, dioxins, PCBs, mirex, kepone and DDT; xLech et al. 1982).

Nevertheless, it has been used effectively in both marine and freshwater studies in which elevated MFO activity reflects elevated PAH or petroleum hydrocarbon pollution (NRCC 1985). A recent study (Luxon et al. 1987) conducted on lake trout (Salvelinus namaycush) throughout the Great Lakes indicated specimens retrieved from the .MT

western end of Lake Ontario exhibited MFO levels six to sixty-two fold higher than fish retrieved from the vicinity of Thunder Bay or Whitefish Bay (Lake Superior), Grand Bend (Lake Huron) or Lake Opeongo (Algonquin Provincial Park, Ontario). They noted that while certain MFO-inducing contaminants (e.g. DDT, PCB) increase gradually from upper to lower lakes, lake trout MFO activity exhibited a sudden increase in Lake Ontario. It was suggested that this may be due to attainment of a threshold inducing concentration of a specific contaminant or group of contaminants (e.g. PAH).

Application of the MFO assay described above is therefore useful in assessing large geographical areas for comparisons between pristine and organically contaminated conditions. However, they require follow-up biochemical and environmental analyses to identify the problematic contaminants. In addition, measurement of MFO activity provides no information on direct biological impacts of the associated contaminants. Since metabolism of PAH to carcinogenic intermediates is mediated through this enzyme system, it could be a valuable aid in correlating frequency of neoplasms with environmental contamination.

Presently, the most pertinent issue regarding MFO activity in fish and other organisms is its role in metabolizing PAH. The action of MFO can either detoxify PAH or produce more reactive intermediates which are cytotoxic and possibly carcinogenic. Figure 2.1.8 relates Phase I reactions (MFO system) to Phase II reactions (conjugation) and illustrates the pathways and intermediate compounds involved in forming the generally reactive diol epoxides of benzo[a]pyrene, which may react with macromolecules including DNA, RNA and various proteins.

There are several enzymes which make up the mixed function oxidases, and not all are necessarily active in a given organism, nor do they cause the same reactions with all PAH. It is therefore of interest to know which PAH have been documented in inducing specific MFO enzymes, and this has been summarized in Table 2.1.14.

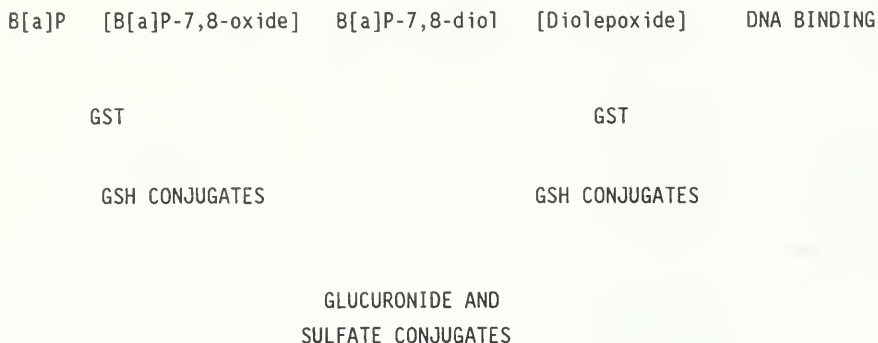


Figure 2.1.8. Activation and detoxification pathways for benzo[a]pyrene.

Footnote: GST = Glutathione-S-Transferase

(After Varanasi et al. 1987)

Clearly, a diverse group of fish possess the capacity to metabolize various PAH. This suggests a wide capability for metabolism of parent PAH to potential carcinogens. However, MFO induction and transformation to a proximate carcinogen is only one factor in the development of neoplasia. Variation in tumorigenic sensitivity is also a function of other factors such as DNA repair efficiency (Varanasi et al. 1987).

It is clear from the mammalian literature that the metabolic profile of a PAH may change considerably depending on the MFO inducing compound. Such an effect takes on added relevance due to differences in reactivity between the metabolites and the parent compounds, and also among metabolites themselves.

TABLE 2.1.14
EFFECT OF SOME AROMATIC HYDROCARBONS ON MFO ACTIVITY IN FISH

Chemical	Test Organism	AH ^a	Effect on Activity			Reference
			ECOD ^b	EROD ^c	BEND ^d	
Benz[f]anthracene	Rainbow trout ^f <u>Salmo gairdneri</u>	+	N.D.	N.D.	N.D.	Statham <u>et al.</u> 1978
7,12-Dimethylbenz [a]anthracene	Rainbow trout ^e <u>Salmo gairdneri</u>	--	N.D.	N.D.	N.D.	Gerhart and Carlson 1978
Benzo[b]triphenylene	Sheepshead ^h	+	+	+	--	James and Bend 1980
	Little Skate ^h	+	+	+	--	James and Bend 1980
Chrysene	Rainbow trout ^e <u>Salmo gairdneri</u>	+	N.D.	N.D.	N.D.	Gerhart and Carlson 1978
3-Methylcholanthrene	Northern pike ^e <u>Esox lucius</u>	+	N.D.	N.D.	N.D.	Balk <u>et al.</u> 1982
	Rainbow trout ^e	+	N.D.	N.D.	N.D.	Singh <u>et al.</u> 1985
	<u>Salmo gairdneri</u>	+	N.D.	N.D.	N.D.	Statham <u>et al.</u> 1978
		+	N.D.	+	N.D.	Pederson <u>et al.</u> 1976
	Brook trout ^f	N.D.	+	N.D.	N.D.	Willis <u>et al.</u> 1980
	<u>Salvelinus fontinalis</u>	+	+	N.D.	N.D.	Addison <u>et al.</u> 1978
	Steelhead trout ^h <u>Salmo gairdneri</u>	+	N.D.	N.D.	N.D.	Pederson <u>et al.</u> 1974
	Mulle ^h <u>Mulgil cephalus</u>	+	N.D.	+	N.D.	Schoor and Srivastana 1984
	Carp ^h <u>Cyprinus carpio</u>	+	--	+	--	Melancon and Leech 1979
	Little Skate ^h	+	+	+	+	James and Bend 1980
	Southern flounder ^h	+	+	N.D.	--	James and Bend 1980
	Atlantic stingray ^h	+	+	N.D.	--	James and Bend 1980

TABLE 2.1.14 (continued)

Chemical	Test Organism	AH ^a	Effect on Activity			Reference
			ECOD ^b	EROD ^c	BEND ^d	
3-Methylcholanthrene (cont'd)	Scup ⁱ	+	N.D.	N.D.	N.D.	Stegeman 1977
Fluoranthene	Rainbow trout ^h <u>Salmo gairdneri</u>	--	N.D.	N.D.	N.D.	Gerhart and Carlson 1978
Pyrene + Fluoranthrene	Rainbow trout ⁱ <u>Salmo gairdneri</u>	--	N.D.	N.D.	N.D.	Gerhart and Carlson 1978
Naphthalene	Rainbow trout ^e <u>Salmo gairdneri</u>	--	N.D.	N.D.	N.D.	Gerhart and Carlson 1978
Beta-Naphthylflavone	Rainbow trout ^e <u>Salmo gairdneri</u> ^e	+	N.D.	N.D.	N.D.	Statham <u>et al.</u> 1978
		+	+	+	N.D.	Andersson <u>et al.</u> 1985
Phenanthrene	Rainbow trout ^e <u>Salmo gairdneri</u>	--	N.D.	N.D.	N.D.	Gerhart and Carlson 1978
	Cunner ⁱ	--	N.D.	N.D.	N.D.	Gerhart and Carlson 1978
Phenobarbital	Rainbow trout ^e <u>Salmo gairdneri</u>	--	N.D.	N.D.	N.D.	Forlin 1980
	Mullet ^e <u>Mulgil cephalus</u>	+	N.D.	N.D.	N.D.	Schoor and Srivastava 1984
Pyrene	Rainbow trout ^h <u>Salmo gairdneri</u>	--	N.D.	N.D.	N.D.	Gerhart and Carlson 1978
	Cunner ⁱ	--	N.D.	N.D.	N.D.	Payne and Penrose 1975
Benzo[a]pyrene	Channel catfish ^e <u>Ictalurus punctatus</u>	+	+	N.D.	N.D.	Fingerman <u>et al.</u> 1983
	Rainbow trout ^e <u>Salmo gairdneri</u> ⁱ	+	N.D.	N.D.	N.D.	Gerhart and Carlson 1978
		+	N.D.	N.D.	N.D.	
	Carp ^e <u>Cyprinus carpio</u>	+	N.D.	N.D.	N.D.	Kurelec <u>et al.</u> 1979
Dibenzothiophene	Cunner ⁱ	--	N.D.	N.D.	N.D.	Payne and May 1979

Footnotes: a - Arylhydrocarbon (benzo[a]pyrene) hydroxylase

b - Ethoxycoumarin-O-deethylation

c - Ethoxyresorufin-O-deethylation

d - Benzphetamine-N-demethylation

e - i.p.

f - Forced feed in gelatin capsule

g - Not specified

h - i.p. or p.o.

i - in water

N.D. - Not detected

+ - Increase

- - No effect or decrease

(Modified from: Lech et al. 1982)

Fish generally exhibit a mode of PAH metabolism which is equivalent to that induced by 3-methylcholanthrene. This is also the P₁-450 isozyme discussed by Lech and Vodcnik (1985). The principal metabolites produced by fish MFOs and their relative proportions are presented in Table 2.1.15. Benzo[a]pyrene has frequently been utilized as a model compound, both as an inducer of MFO activity as well as a substrate. From the numerous studies it appears that hydroxy forms of B[a]P and quinones account for about 50% of the total B[a]P metabolites while diols represent approximately the other half. It is significant that B[a]P 7,8-diol is the major diol observed in both fish species and the rat indicating that metabolism is comparable among fish and laboratory mammals and that a carcinogenic electrophile is produced in fish following exposure to B[a]P and metabolism by the MFO system.

The higher proportion of B[a]P 7,8-diol is significant because of its ability to produce adducts by binding covalently with DNA, RNA and proteins. In the mammalian literature, the formation of adducts is considered to be the first step in the initiation of a series of events leading to tumor formation (Miller 1970, Miller and Miller, 1981).

Several other concerns of PAH contamination are also associated with MFO induction. The existence of basal levels and induction of MFO activity during the embryonic period of development should render this life stage particularly sensitive to teratogenic and embryotoxic effects of PAH since the electrophilic intermediates would interact with rapidly proliferating tissues. Embryonic MFO activity has been reported for the brook trout (Salvelinus fontinalis) (Binder and Stegeman 1983) and in rainbow trout (Salmo gairdneri, Hannah et al. 1982). Embryo toxicity and teratogenic effects were also noted in the latter study. This concept is also consistent with the high PAH toxicity and teratogenicity observed for avian embryos, which have unusually high basal MFO activity (section 3.2.2.2.1).

TABLE 2.1.15
METABOLITES OF PAHS OBSERVED IN VARIOUS TISSUES OF FISH
FOLLOWING IN VIVO EXPOSURE

Chemical	Test Organism		Proportion (%) of Observed Metabolites						Quinones	Reference
			1-OH	3-OH	9-OH	4-5-Diol	7,8 Diol	9,10 Diol		
Benzo[a]pyrene	Coho Salmon ^{1,8}	I	16.0	43	4.2	NM	24	12		Krahn <u>et al.</u> 1981
	<u>Oncorhynchus kisutch</u>									
	English Sole ¹									
	<u>Parophrys vetulus</u>	U	2.3	26	2.3	1.1	26	17	7	Varanasi <u>et al.</u> 1986
		I	1.0	26	1.0	1.3	26	20	6.7	
	Starry Flounder ¹									
	<u>Platichthys stellatus</u>	U	19	20	5	3.3	22	24	7	Varanasi 1986
		I	9.8	22	0.8	4.1	27	26	9.6	
	Southern Flounder ¹	U	—	28	10	3.6	28	22	10	
		I	—	13	7	2.6	29	24	11	
<u>DMN 2-Methanol</u> - <u>3-Hydroxy DMN</u>										
Dimethylnaphthalene	Coho Salmon		69.5		30.5					
	<u>Oncorhynchus kisutch</u>									

TABLE 2.1.15 (cont'd)

Chemical	Test Organism	1-naphthol	2-naphthol	1-2-dihydrodiol 1-2 dehydroxynaphthol	1-naphthyl glucuronic	1-naphthyl sulfate	1-naphthyl glucoside	Unidentified	Reference
Naphthalene	Coho Salmon ¹	1.2	NM	67	1	1	0.5	290	Collier <i>et al.</i> 1978
	<u>Oncorhynchus</u> ²								
	<u>kisutch</u>	10.3	NM	68	6.8	7.2	1.6	517	Collier <i>et al.</i> 1978
	Rainbow Trout ¹	0.7	0.7	48.2	trace	24.5	NM	25	Collier <i>et al.</i> 1979
	<u>Salmo gairdneri</u> ^{3,4}	--	--	86	--	--		7	Collier <i>et al.</i> 1979
		--	--	74	0.8	18.8	NM	6.2	Collier <i>et al.</i> 1979
	Rainbow Trout ^{5,6}	32.2	NM	NM	32	11-21	NM	NM	Krahn <i>et al.</i> 1980
	<u>Salmo gairdneri</u>								
<u>DIOL</u>									
	Coho Salmon ¹	40	NM	11	25	2	A	22	Roubal <i>et al.</i> 1977
	<u>Oncorhynchus</u> ²	7	NM	7	75	2	A	9	
	<u>kisutch</u> ³	32	NM	5	35	17	A	11	
	⁶	61	NM	26	13	ND	A		
	⁷	47	NM	7	14	26	A	6	

Footnotes: 1 - liver
 2 - gall bladder
 3 - brain
 4 - blood
 5 - bile
 6 - heart
 7 - flesh
 8 - this study was conducted in vitro, all others in vivo
 U - uninduced
 I - induced
 ND - not detected
 NM - not mentioned
 A - 1-naphthyl sulfate and 1-naphthyl glucoside were not differentiated

The potential also exists for reproductive effects to occur as a result of induced MFO activity. Some MFO enzymes actively metabolize corticosteroid hormones which regulate spawning behavior and physiology. During spawning, it has been noted that MFO activity declines and this is believed to facilitate the maintenance of higher levels of corticosteroid hormones during the reproductive phase. This concept is consistent with observations in laboratory mammals (Lech et al. 1982), and MFO activity in feral lake trout (Luxon et al. 1987). If high levels of PAH induce MFO activity to relatively high levels prior to and during the spawning phase, this may impair the levels of corticosteroids which are critical during this period. The same concept also applies to any inducer of the MFO system (e.g. PCBs, TCDD, Mirex), however, as yet the hypothesis has not been investigated.

2.1.2.3 Chronic Effects

2.1.2.3.1 Carcinogenesis/Tumor Induction

Various PAH have been recognized as carcinogenic to mammals for some time. Most carcinogenic PAH consist of 4-, 5-, or 6-rings, and are quite angular in structure (e.g. possessing bay regions). DePierre and Ernster (1978) demonstrated PAH undergo metabolic activation via the MFO system to form carcinogenic reactive electrophilic metabolites. Linear or condensed PAH ring structures are less likely transformed to carcinogens. It is well known that the MFO-cytochrome P-450 system of fish can produce carcinogenic or mutagenic metabolites in vitro and in vivo (Varanasi et al. 1987, Balk et al. 1982, Lech et al. 1982 and others). Many carcinogenic chemicals are also mutagenic or teratogenic, and carcinogenicity is inferred from data demonstrating mutagenicity or teratogenicity, since this can be determined more rapidly. Considerable interest in tumor induction in fish has been generated both as a direct concern for aquatic environments and as a possible sentinel of contaminant effects on the water and food supply for human use (Black, 1985).

While there have been many studies implicating PAH compounds as a causative agent of fish tumors, only some of the more recent studies have conclusively demonstrated cause-effect relationships. Many field studies have compared tumor frequencies in fish populations from "contaminated" sites and "clean" sites. Any elevation in tumor frequency has been associated with a host of contaminants found to be present at the polluted site, which typically includes various PAH (Black 1982, 1983b, Black et al. 1980, 1981, 1985, Bauman and Harshbarger 1985, and Harshbarger et al. 1984). While such studies should be of concern, they do not clearly implicate any one factor, including PAH compounds, as being a causative agent. These studies do not form any type of exposure-response relationship which could be demonstrated by relating tumor frequency to age or sex of fish or by sampling along a gradient of pollution. Relating a factor such as age to tumor frequency could provide a useful substitute for a dose response relationship.

Among the most informative field studies published to date are those conducted by Malins and co-workers in Puget Sound, Washington. For example, Krahn et al. (1986) examined the relationship between the level of sediment contamination, PAH metabolite levels in bile, and hepatic lesions of English sole (Parophrys vetulus) at eleven sites. They found a strong positive correlation between benzo[a]pyrene metabolite equivalents in bile (mean range: 67-2100 $\mu\text{g/kg}$ bile wet wt.) and the prevalence of hepatic lesions (range: 6.2-90%). Weak correlations were made between lesion frequencies and levels of sediment contaminants which included naphthalenes, phenanthrenes, and benzo[a]pyrene. The prevalence of hepatic neoplasms in this species was also demonstrated to increase with age (Landolt et al. cited in Varanasi et al. 1987). However, this trend is believed to reflect the time required after initiation for tumors to be discernibly manifested, rather than a function of age susceptibility. Other single site studies by Malins et al. (1985a, 1985b) showed elevated hepatic neoplasms in association with sediments heavily contaminated by PAH. Benthic food organisms in fish stomachs were also heavily

contaminated, demonstrating a food chain transfer of PAH from sediments to benthic fish. This demonstrates the utility of selecting a benthic feeding fish species for monitoring contaminant effects.

Neff (1979) found no reports of tumor induction in aquatic organisms exposed to environmentally realistic levels of PAH via water, food or sediments. More recent studies, using novel and sensitive techniques such as micro-injection of PAH into fish eggs (Metcalf and Sonstegard, 1984) have induced tumor formation at realistic exposure levels. While many laboratory studies have used excessively high exposure levels of PAH to induce tumors, they remain useful since exposures are typically brief relative to exposures in the environment. The delay time from initiation to tumor development is a limiting factor in laboratory studies since examination for tumors is typically conducted after only one year. Longer exposures, for example five to ten years, may evoke higher tumor frequencies at lower concentrations. However, such studies are clearly not practical due to time constraints.

Black (1983a) combined field studies with laboratory exposures to extracts from contaminated river sediments of Eastern Lake Erie and the Upper Niagara River. Laboratory dosing of different sediment extracts high in PAH content did not attempt to duplicate field exposures since dosing involved painting the extracts onto the upper lip of brown bullheads. After 18 months of near weekly dosings, five of twenty-two surviving fish showed epidermal hyperplasia and papillomas similar to those noted in feral fish found in polluted areas near Buffalo, N.Y. None of the control fish exhibited that response.

An eighteen month long study by Hendricks et al. (1985) examined the effects of benzo[a]pyrene exposures to rainbow trout on MFO induction and tumor induction. B[a]P was administered either in the diet (500, and 1000 mg/kg) or as weekly intraperitoneal injections (1 mg/dose). MFO enzyme activity significantly increased with dose and exposure

duration. Liver neoplasia frequency also increased with exposure time (15% after 12 months to 25% after 18 months) in fish fed 1000 mg/kg B[a]P. Fifty percent of fish injected with B[a]P developed hepatocellular neoplasms.

Schultz and Schultz (1982) demonstrated that repeated short term aqueous exposures of two species of viviparous fish (Poeciliopsis lucida and P. monacha) to 5 mg/L 7,12-dimethylbenz(a)anthracene (DMBA) induced hepatocellular neoplasms in 47 out of 106 fish after 6-9 months of exposure. DMBA is also widely known to be a carcinogen in mammals. Short exposures to 0.25 mg/L DMBA failed to induce neoplasms, suggesting that there is some threshold level of exposure required. This work contradicts earlier work by Pliss and Khudoley (1975) where no tumor induction resulted from DMBA or 3-methylcholanthrene in the guppy (Poecilia reticulata).

Recent and preliminary work by Hawkins et al. (1988) provides useful data concerning the induction of fish neoplasms as a result of environmentally realistic concentrations of model PAH. In these studies, guppies (Poecilia latipes) were exposed to concentrations of B[a]P or DMBA of less than 5 µg/L, 30-50 µg/L and 150-250 µg/L derived from carrier-mediated (dimethylformamide) and glass fiber (0.45 µM) filtrate. Two 6-hour exposures, one week apart (i.e. pulse dosed), conducted on young specimens resulted in hepatic neoplasms and pre-neoplastic lesions in both species for both carcinogens.

Preliminary analyses indicated B[a]P-induced neoplasms in the guppy were limited to the high exposure group with incidences of approximately 10% and 24% at 24 and 36 weeks post initial exposure, respectively. DMBA was found to be much more carcinogenic to both fish than B[a]P with neoplasms in guppies occurring at both the intermediate and high concentrations (10% to 66%) and at all three concentrations in the case of medaka (2.5% to 51%). In addition, medaka developed numerous non-hepatic lesions. While these preliminary results do not address fish species indigenous to Ontario,

they are important because they demonstrate carcinogenic effects from waterborne exposures in the parts per billion range. In addition, because neoplasms induced by B[a]P in the guppy and non-hepatic neoplasms induced by DMBA in the medaka were limited to the high exposure concentrations (which contained particulates), it suggests that the insoluble or particulate fraction of PAH plays an important role in carcinogenesis (Hawkins et al. 1988). However, in terms of a possible threshold concentration, it is difficult to extrapolate the lack of tumor induction at the $< 5 \mu\text{g/L}$ concentration/exposure regimen, to wild fish populations involving other species and the chronic lifetime exposures that may occur in some polluted environment.

A novel technique for tumor induction was developed by Metcalfe and Sonstegard (1984) in which micro-injections ($0.5 \mu\text{L}$) of dissolved carcinogens were implanted into the perivitelline space of rainbow trout embryos at the eyed stage of development. Micro-injections were facilitated through the use of modified glass capillary micropipettes (diameter approximately $150 \mu\text{m}$) and a micromanipulator (Figure 2.1.9).

In certain cases a two hour, pre-injection incubation of the PAH with rat liver microsome was used to promote MFO metabolite formation. Embryos were then allowed to develop through to one year old juveniles, at which time they were sampled for overt tumors and neoplastic lesions. Injections of DMBA ($0.25 \mu\text{g/embryo}$) produced grossly visible neoplasms, eosinophilic nodules and carcinomas to a maximum frequency of 6.3% ($n=32$) when rat liver microsomes (S-9) were employed, and to 3.5% ($n=28$) when S-9 was omitted. Similar results were found by Black et al. (1985) in which essentially the same technique was used to investigate the tumorigenicity of B[a]P to rainbow trout. After nine months following injections of $10 \mu\text{g/embryo}$ (136.1 mg/kg) a tumor incidence of 8.7% ($n=23$) was observed while all control fish appeared healthy ($n=48$).

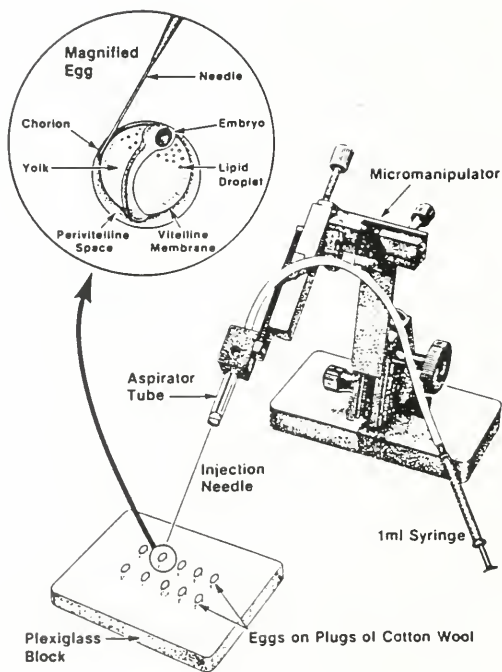


Fig. 2.1.9 Apparatus used in embryo injection assay and a magnified trout embryo showing the position of the needle at injection.

Currently, this technique has also been employed to assess the carcinogenic hazard of various lake sediments in Ontario (Metcalf, personal communication, Trent University, Ontario). In these studies extracts of sediment samples from Oakville Creek, South Baymouth and Hamilton Harbour were injected into rainbow trout sac fry and necropsied after twelve months. Preliminary results revealed no carcinomas associated with either Oakville Creek or South Baymouth sediments, but a significant incidence (8.9%) resulted from the use of Hamilton Harbour sediment extract, suggesting that an elevated carcinogenic risk exists for aquatic organisms exposed to this sediment. Further refinement of the technique and its inherent variation is desirable before results are used in quantitative risk assessment, however.

Analysis of extracts from all three sites is summarized in Table 2.1.16 and reveals a substantially elevated content of PAH in Hamilton Harbour extract compared to the other sites. Using calculations based on the extract concentration and technique described by Metcalf and Sonstegard (1984) and omitting the contributions of anthracene and benzantracene as noted in Table 2.1.16, the total quantity of PAH injected per embryo for Oakville Creek, South Baymouth and Hamilton Harbour extracts was 0.563, 0.026 and 169.67 ng/embryo, respectively. These calculations, based on the sediment extract analysis and technique employed support the incidence of tumors observed in the study and the concept that sediments from Hamilton Harbour may pose a significant carcinogenic risk to aquatic organisms indigenous to this area.

TABLE 2.1.16
PAH ANALYSIS OF SEDIMENT EXTRACTS USED IN MICROINJECTIONS
OF RAINBOW TROUT EMBRYOS (ng PAH/g extract)

PAH	Oakville Cr.	South Baymouth	Hamilton Harbour
Naphthalene	1.35	ND	2030
Acenaphthylene	0.55	ND	334
Acenaphthene	0.52	ND	398
Fluorene	2.62	ND	1390
Phenanthrene	35.00	1.83	11400
Anthracene	1.72	ND	A
Fluoranthene	29.70	0.76	5060
Pyrene	24.70	0.81	4260
Chrysene	8.20	ND	2360
Benz[a]anthracene	8.20	1.80	B
Benzo[b]fluoranthene	ND	ND	3490
Benzo[k]fluoranthene	ND	ND	685
Benzo[a]pyrene	ND	ND	4780
Indeno[1,2,3-c,d]pyrene	ND	ND	387
Dibenzo[a,h]anthracene	ND	ND	320
Benzo[ghi]perylene	ND	ND	1600

Footnotes: A - Phenanthrene & Anthracene Peaks Merged
B - Chrysene & Benz[a]anthracene Peaks Merged
ND- Not Determined

(Data calculated from Metcalfe, unpublished)

2.1.2.3.2 Histopathology

Quality studies on sublethal histopathological effects of chronic, controlled exposure to PAH are few. Most available studies involving fish use exposures which are acutely lethal. Histological studies of tissues responding to PAH exposure have been summarized in Table 2.1.17.

Recent studies involving laboratory exposures of resident fish species of Ontario to PAH are limited. Hose et al. (1984) exposed rainbow trout eggs to a range of B[a]P concentrations and followed development from fertilization until 36 days post-hatch. At low concentrations (0.08 $\mu\text{g/L}$) some nuclear pycnosis was prevalent in the brain and neural retina of alevins, and some abnormal erythrocytes were evident. However, at concentrations of 0.21 $\mu\text{g/L}$ and higher, there was an increase in ocular, neural, and hematological effects. The effects were observed in tissues marked by the highest levels of B[a]P uptake (Hannah et al. 1982).

Stoker et al. (1985) exposed rainbow trout and brown bullheads for 16 h to very high concentrations of water soluble fractions of PAH extracted from coal and sediment. Examination of liver and gill tissue demonstrated that rainbow trout were consistently more sensitive than brown bullheads. The major effects were hepatocyte disruption and mitochondrial swelling along with fusion, clubbing and hyperplasia of gill lamellae. No dose-response data was provided and changes were not surprising in view of the high concentrations the fish were exposed to, and the acute time frame.

Chronic histological effects of PAH exposure are usually related to increased hepatic activity associated with PAH metabolism and to a decreased feeding response. Although Hawker et al. (1980) did not note a difference in food consumption of control and PAH diets, other studies have reported a decrease in feeding rates of exposed fish (Fletcher et al. 1982).

TABLE 2.1.17
HISTOPATHOLOGICAL RESPONSES OF FISH EXPOSED TO PAH COMPOUNDS

A. FRESHWATER FISH - LAB EXPOSURES

Species	PAH	Exposure Concentration	Method	Duration	Tissues	Results	Reference
Rainbow trout (<u>Salmo gairdneri</u>)	Benzo(a)pyrene	Series 0-2.99 ng/ml in water	on coarse sand under water	fertilization to 36 d post-hatch	blood, whole body	-looked at deformities -LOEC : 0.21 µg/ml in water.	Hose <u>et al.</u> , 1984
Rainbow trout	Solvent-refined coal extract and Black River sedi- ment extract	10 µg/L (in acetone)	waterborne exposure in 3.5 l flasks	16 hr exposure to 8-11 cm trout	liver gill	-trout more sensitive than bullhead -effects on liver & gill	Stokes <u>et al.</u> , 1985
Rainbow trout	Benzo(a)pyrene	250 ng	microinjection	12 months	liver	-eosinophilic nodules	Metcalfe <u>et</u> Sonstegard 1984
Brown bullheads (<u>Ictalurus nebulosus</u>)	Solvent-refined coal extract and Black River sedi- ment extract	10 µg/L (in acetone)	waterborne exposure in 3.5 l flasks	16 hr exposure to 8-11 cm trout	liver gill	-trout more sensitive than bullhead -effects on liver & gill	Stokes <u>et al.</u> , 1985
Brown bullheads	PAH sediment extract	280 mg PAH/ml	Skin painting	18 months	epidermis	-irregular hyperplasia	Black <u>et al.</u> , 1985
Chinook salmon (<u>Oncorhynchus tshawytscha</u>)	mixed hydro- carbons with & without chlorinated biphenyls ^a	5 mg/Kg	dietary	28 d uptake and 21 d depuration	intestinal mucosa	-cellular inclusions	Hawkes <u>et al.</u> , 1980

B. FRESHWATER FISH - FIELD COLLECTION

Species	Collection Site	Exposure Duration	Tissue	Results	Reference
Black bullhead (<u>Ictalurus nebulosus</u>)	Collected from a 2 acre final oxidation sewage pond with oxygenation and chlorination	3 - 4.5 months and longer	liver size and enzymes	liver dysfunction and mouth papillomas	Tan <u>et al.</u> , 1983
Silver carp (<u>Hypophthalmichthys molitrix</u>)					

TABLE 2.1.17 (continued)
HISTOPATHOLOGICAL RESPONSES OF FISH EXPOSED TO PAH COMPOUNDS

C. MARINE FISH - LAB EXPOSURE

Species	PAH	Exposure Concentration	Method	Duration	Tissues	Results	Reference
Mummichog (<u>Fundulus heteroclitus</u>)	Naphthalene	30 to 0.002 µg/L	water	1 to 15 d	screening most tissues	threshold for 15 d study .02 to .002 µg/l	DiMichele & Tayl 1978
Mullet (<u>Mugil cephalus</u>)	3-methylcholanthrene	30 mg/kg	injection	single	liver	by 7 d histologic evidence of increased MFO activity	Schoor and Couch 1979
Winter flounder (<u>Pseudopleuronectes americanus</u>)	Venezuelan Crude Oil	3000 ppm	applied to sand	17 - 10 weeks in 3 sessions	liver	most effects related to increased hepatic activity and decreased feeding	Fletcher <u>et al.</u> , 1982
Cunners (<u>Tautoglabrus adspersus</u>)	Venezuelan Crude Oil	150 ml to 240 L tanks	applied to water surface	6 months	many tissues	decreased testes weight	Payne <u>et al.</u> , 19

D. MARINE FISH - FIELD COLLECTION

Species	Collection Site	Exposure Duration	Tissue	Results	Reference
Plaice (<u>Pleuronectes platessa</u>)	Collected during five different times from site of oil spill (mixed Iranian and Arabian)	2 years	many tissues	Many changes associated with tissues involved with uptake and depuration	Baensley <u>et al.</u> , 1982

Footnote: 1 - Chlorinated biphenyls = mixture of 2
chlorobiphenyl, 2,2' dichloro-, 2,5,2' trichloro-, and 2,5,2,'5' tetrachlorobiphenyl

Hydrocarbon mixture = benzothiophene, methyl-naphthalene, dimethyl- and trimethyl-naphthalene, fluorene, phenanthrene,
1-phenyl-dodecane and heptadecylcyclohexane

Chinook salmon were exposed to a PAH-contaminated diet, with and without concomitant exposure to dietary chlorinated biphenyls for 28 days (Hawkes et al. 1980). They documented the presence of finely granular, floccular material in intestinal mucosal cells, and found that the additional stress of organochlorine exposure increased intestinal damage. The damage included exfoliation and sloughing of intestinal cells. Despite the obvious indications of histological intestinal differences, no effect on weight gain was detected, and therefore no distinct impairment could be suggested.

When direct effects of PAH exposure are evident, they are usually associated with an increase in MFO activity (Schoor and Couch 1979, Fletcher et al. 1982, Tan et al. 1983), or appear in tissues associated with PAH uptake and depuration (Hannah et al. 1982, Haensley et al. 1982). Naphthalene was selectively accumulated by gills, liver, gut tissue and the gall bladder (DiMichele and Taylor 1978), and by muscular and nervous tissue (Haensley et al. 1982, Hannah et al. 1982, DiMichele and Taylor 1978).

Haensley et al. (1982) collected fish over a period of two years from an area exposed to an extensive spill of mixed Arabian and Iranian crude oil. Histological changes were associated with a decreased feeding activity, abrasive effects (gill) and excretory activity (liver, kidney). Changes included hyperplasia and hypertrophy of gill lamellar cells, gastric gland degeneration, an increase in melanomacrophage activity and a decrease in hepatocyte vacuolation (lipid).

Chronic laboratory exposure to crude oil was not associated with any major alterations (Fletcher et al. 1982) but DiMichele and Taylor (1978) found gill hyperplasia after a 15 days exposure to naphthalene concentrations as low as 2 $\mu\text{g/L}$. They also found pancreatic, hepatic and brain ischemia resulting from exposures to 2 to 30 ppm.

The major role of PAH in chronic histological damage seems to be related to an interaction of the PAH molecule with cellular membranes (Neff 1985). Such an interaction is consistent with blood stasis noted

in other studies (DiMichele and Taylor 1978, Hose et al. 1984) and may be associated with an increased rigidity of erythrocytic membranes.

2.1.2.3.3 Growth and Reproductive Effects

In addition to the sublethal effects discussed thus far, PAH may influence growth, reproduction and chronic survival. This group of response variables is conventionally utilized to determine chronic values for toxicants since, by the nature of the effect and exposure duration, they provide a sensitive whole organism measure of the integrated impacts. While the same may be true of certain other response variables (e.g. carcinogenesis), they have been addressed separately due to a more defined mode of action.

Growth

Studies suitable for the development of "fish chronic toxicity values" as defined by Stephan et al. (1985) were limited to only two compounds, naphthalene and acenaphthene (Table 2.1.18). For each of the studies pertaining to either compound, good agreement was observed. Chronic values for both compounds range from 0.405 to 0.710 mg/L, and one should note the relative close proximity to the acute lethal toxicity observed for these compounds (Table 2.1.1). This suggests the concentration range spanning acute and chronic effects is relatively low (Kenaga 1982).

In both cases depressed growth was a sensitive measure of impact. Poor growth has also been observed in rainbow trout exposed to benzo[a]pyrene (Hendricks et al. 1985) and in bluegill sunfish exposed to fluorene (Finger et al. 1985). In the former case, dietary B[a]P at 1000 mg/kg wet weight reduced final wet wt by 15% over an 18 month exposure. The depressed growth was partially explained by an increase in the feed:gain ratio from 1.14 (controls) to 1.32 (exposed). Hepatocarcinomas also resulted from this exposure.

TABLE 2.1.18
SUBLETHAL CHRONIC VALUES FOR FISH

Chemical	Test Organism	Method	Concentration Limits (mg/L)	Chronic Value (mg/L)	Acute: Chronic Ratio	Reference
Naphthalene	Fathead Minnow <u>Pimephales promelas</u>	EL	0.450-0.850	0.620	3.2 ^a	DeGraeve <u>et al.</u> 1980
	Coho Salmon <u>Oncorhynchus kisutch</u>	ELS	0.378-0.672	0.577	3.6	Moles <u>et al.</u> 1981
Acenaphthene	Sheepshead Minnow <u>Cyprinodon variegatus</u> (marine)	EL	0.520-0.970	0.710	3.1	U.S. EPA 1978
	Fathead Minnow <u>Pimephales promelas</u>	ELS	0.332-0.495	0.405	1.5	Cairns & Nebeker 1982

Footnotes: a - Ratio based on acute 96-h LC50 of 1.99 mg/L as noted in Table 2.1.1.

EL - Embryo-larval study

ELS - Early life cycle study

Fluorene, at a nominal aqueous exposure of 0.25 mg/L caused a somewhat larger reduction in wet weight of bluegill sunfish following a 30-day exposure, 25% vs. control (Finger et al. 1985). Relative to the measured 96-h LC50 in this study, the nominal chronic value (0.177 mg/L) yields an acute to chronic ratio of 5.14. The reduced growth rate in this study was primarily due to a reduced food consumption.

Hose et al. (1981) reported a reduction in size of yolk sac reserve in flathead sole larvae (*Hippoglossoides elassodon*) following maternal exposure to dietary B[a]P (8.0 mg/kg body wet weight). Although growth effects were not addressed in the study, it is likely that reduced endogenous energy reserves would limit growth potential. This is particularly important for developing larvae since failure to achieve a sufficient size may impair the transition to exogenous feeding and therefore, survival.

Reproduction

Documentation of PAH impacts on reproduction is limited, although several inferences may be made from indirect evidence. For example, Hose et al. (1981) clearly demonstrated that embryos are subject to exposure via maternal transfer. Some of the ensuing terata (see section 2.1.2.2.1) would lead to the early life stage mortalities and therefore reduced recruitment. However, in addition to post hatch mortalities, hatching success was reduced to only 12% in comparison to 57% for controls.

It is well known that the MFO system, in addition to detoxifying xenobiotics, is also involved in metabolism of reproductive hormones (Lech et al. 1982). Induction of MFO activity has been shown to reduce some of these hormones, however, solid evidence of MFO induction compromising spawning activities is limited. Spies et al. (1985) reported a weak inverse correlation between arylhydrocarbon hydroxylase activity and fertilization success in starry flounder collected from areas high in PCB contamination ($r=-0.60$, $p=0.001$).

Impacts on reproduction may, however, be mediated through more direct mechanisms. For example, metabolism of benzo[a]pyrene to reactive metabolites and their subsequent covalent binding to gonadal macromolecules, including testicular DNA, has been reported for English sole, Parophrys vetulus (Varanasi et al. 1982a). There is therefore, potential for impairment of developing spermatozoa in vivo, which has implications on fertilization success as noted by Spies et al. 1985).

Effects of a mixture of organic and inorganic chemical contaminants on fertilization, hatching success and prolarval survival of striped bass (Morone saxatilis) was reported by Hall et al. (1984). The stock mixture included perylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene and chrysene (each at 40 ng/L). No significant effects on fertilization or hatching success were observed following a 24 to 48-h exposure. However, significant reduction in survival was observed after 144 h continuous exposure to the stock mixture. Thus short term studies of a reproductive nature may not be a sensitive test for certain species and/or PAH.

2.1.2.4 Toxicokinetics

Because polycyclic aromatic hydrocarbons are hydrophobic, and therefore lipophilic, their chemical properties (octanol-water partition coefficient - K_{ow} , water solubility, etc.) favour their partition into biota from water. PAH, however, do not follow the Quantitative Structure Activity Relationships (QSAR) for bioconcentration of organic contaminants into fish (see Veith et al., 1979). This is primarily due to their active metabolism by the MFO system, leading to significant reductions in their apparent bioconcentration. Most models of bioaccumulation, and simple models, do not apply when there is a significant level of toxicant metabolism. For comparison, a QSAR model for PAH bioconcentration for invertebrates such as Daphnia pulex works well since they do not metabolize PAH to any significant extent (Hawker and Connell, 1986, Southworth et al. 1980).

Neff (1979) reports that the PAH of greatest aquatic concern are those ranging in molecular weight (MW) from naphthalene (MW 128) to coronene (MW 300). PAH of MW > 300 are sufficiently low in solubility that they are considered to be of little concern with respect to aqueous uptake, but may be ingested when adsorbed to particulates. The influence of complexation on bioavailability is discussed further in section 2.4.1.3. Studies on PAH bioaccumulation by fish have centered on a select few compounds ranging from naphthalene to benzo[a]pyrene.

A major route of low molecular weight PAH uptake (e.g. naphthalene) by fish is through aqueous uptake via the gill (Balk et al. 1984, Lee et al. 1972b, Southworth et al. 1979a). There are no data which specifically address PAH uptake efficiency for gills. Any factors which would affect the rate of respiration or solubility (bioavailability) of PAH would likely affect uptake rates. Several studies have demonstrated that PAH may accumulate through the skin (Varanasi et al. 1978, Balk et al. 1984), but at a far slower rate than via the gill.

The uptake of PAH from food via the gut has been demonstrated for a number of compounds and a range of species. Estimates of assimilation efficiencies of some PAH are given in Table 2.1.19. While estimates of dietary assimilation vary widely for different PAH and species, direct comparisons suggest that dietary uptake of PAH is minor in comparison to direct aqueous uptake (Southworth et al. 1979b).

Recent field studies suggest that sediment may be a major source of PAH contaminant uptake in benthic fish. The problems associated with fish migration and variable sediment contamination, however, make it difficult to reliably correlate the level of PAH accumulation to the level of environmental contamination.

Several laboratory studies conducted by Varanasi and co-workers have demonstrated that both low and intermediate molecular weight PAH (e.g. naphthalene and benzo[a]pyrene) are readily absorbed by English sole from spiked sediments (Varanasi and Gmur 1981a,b, Stein et al. 1984, Stein et al. 1987). An important observation from these studies is

that the presence of PCB's or crude oil in sediments spiked with PAH compounds appears to facilitate the uptake of PAH into fish. Significantly higher levels of PAH compounds were detected in the bile of fish exposed simultaneously to both xenobiotics versus fish exposed to sediments spiked with PAH only. The results also indicate that PAH uptake directly from sediment particles are an important route versus that available in sediment-associated water.

Short-term exposures of English sole to sediment-associated naphthalene, B[a]P and Prudhoe Bay crude oil revealed that naphthalene and metabolites were rapidly concentrated in the liver within 24 hours, but were reduced by approximately 65% within 168 hours. The reduction in tissue naphthalene was believed to be mediated through facile excretion rather than metabolism and depuration. Conversely, the more lipophilic and larger B[a]P did not accumulate as rapidly, however, it continued to increase over the 168-hour exposure period to the extent that liver concentrations had equilibrated with sediment concentrations (Varanasi and Gmur 1981a,b).

Bioaccumulation factors for B[a]P from sediment were 14 for bile, 0.3 for liver and 0.001 for muscle. High levels of radioactivity in the gill suggested that uptake was primarily due to B[a]P being desorbed from the sediment. Although bioaccumulation was low, the high activity in bile (85 - 99% as metabolites) demonstrated that there was significant transfer and cycling of B[a]P through the fish. This is important when the exposure to carcinogenic metabolites are considered.

The determination of bioconcentration factors (BCF) for PAH by fish is difficult due to problems associated with their rapid metabolism and excretion. Many factors can affect the rate of PAH metabolism, making it difficult to define steady state conditions. Literature values of BCF can be difficult to interpret since many studies have used radio-labeled PAH and estimates often include both parent and metabolite

TABLE 2.1.19
ESTIMATES OF PAH ASSIMILATION EFFICIENCIES
FROM THE DIET FOR FISH

Compound	Species	% Assimilation Efficiency	Reference
Fluorene	Rainbow Trout	14	Niimi & Palazzo 1986
Phenanthrene	Rainbow Trout	4	
Anthracene	Rainbow Trout	<1	
Fluoranthene	Rainbow Trout	<1	
Benzo[a]pyrene	Rainbow Trout	<1	
Benz[a]anthracene	Rainbow Trout	<1	
Acridine	Fathead minnow	<1% relative to aqueous uptake	Southworth <u>et al.</u> 1979b
Naphthalene	Starry flounder	4%	Varanasi <u>et al.</u> 1979
Phenanthrene	Rainbow trout	>21.5%	Solbakken & Palmork 1980

compounds. Overall, estimates of PAH bioconcentration factors for fish tend to be low relative to their lipid-water partition coefficients (Southworth et al. 1979b, Spacie et al. 1983), (Tables 2.1.20 and 2.1.21.)

Whole body BCF's do not appear to exceed 700 when only the parent compound is considered. The differences between log K_{ow} based estimates and experimentally derived BCF values may be explained by metabolism of PAH. For example, Southworth et al. (1980) found that PAH bioconcentration in Daphnia pulex was similar to that predicted by the model of Veith et al. (1979), but bioconcentration by fathead minnows was not similar. However, unlike fish, Daphnia show little active metabolism of PAH which suggests that only uptake, and not bioconcentration, of PAH by fish may follow Veith's model because of active metabolism.

All studies examining the depuration or persistence of PAH in fish tissue show that fish rapidly clear their body burden of PAH and metabolites (Table 2.1.22). Half-life values range from 17 h for anthracene to 9 days for phenanthrene. Half-life estimates based on oral administration appear to yield higher estimates when compared to aqueous exposures. Solbakken et al. (1984) showed that benzo[a]pyrene taken up by cod eggs in a 1-day exposure was not depurated effectively until after eggs hatched, suggesting activation of metabolism was a factor. Phenanthrene and naphthalene, however, were depurated as effectively by eggs and larvae. Since most studies of PAH uptake and depuration are of short duration, little consideration has been given to the effects of extended exposure on enzyme induction and PAH excretion rates. While the majority of PAH may be depurated rapidly, small amounts of covalently bound metabolites may present significant impacts (see section 2.1.2.2.2).

TABLE 2.1.20
BIOCONCENTRATION FACTORS OF PAH COMPOUNDS
FOR FISH AND LOG P ESTIMATED BCF
FROM MODEL OF VEITH et al. (1979)

PAH	Species	BCF*	Estimated Duration	Estimated BCF	Reference
¹⁴ C quinoline	Fathead minnow	8(E)	4d	7	Southworth <u>et al.</u> 1980
¹⁴ C acridine	"	105(E)	4d	100	"
¹⁴ C benz[a]- acridine	"	100(E)	4d	approx.1000	"
¹⁴ C dibenz[ah]- acridine	"	80(E)	4d	approx.10000	"
¹⁴ C anthracene (parent compound)	Bluegill Sunfish	675(NE)	5h	1209	Spacie <u>et al.</u> 1983
" (total ¹⁴ C)	"	900(NE)	5h	1209	"
¹⁴ C benzo[a]pyrene (parent compound)	"	490(NE)	5h	28250	"
" (total ¹⁴ C)	"	4900(NE)	5h	28250	"

Footnotes: * (E) - BCF determined at equilibrium
(NE) - non-equilibrium determination of BCF

+ - estimates of BCF for parent compound only and as total ¹⁴C (parent plus metabolite compounds).

TABLE 2.1.21
APPARENT BIOCONCENTRATION FACTORS OF PAH
COMPOUNDS FOR FISH

PAH	Species	BCF	Exposure Duration	Reference
Acridine	Fathead minnow	125(E)	15 d	Southworth <u>et al.</u> 1979
¹⁴ C-anthracene	Rainbow trout	200(NE)	18 h	Linder & Bergman 1984
¹⁴ C-naphthalene	Mummichog (marine)	2.2(NE)	2 h	DiMichele & Taylor 1978
	Atlantic cod egg (marine)	18(NE)	1 d	Solbakken <u>et al.</u> 1984
	yolk-sac larvae	23(NE)	1 d	"
¹⁴ C-phenanthrene	egg	108(NE)	1 d	"
	yolk-sac larvae	223(NE)	1 d	"
	9 d old larvae	427(NE)	1 d	"
¹⁴ C-benzo[a]pyrene	egg	22(NE)	1 d	"
	yolk-sac larvae	80(NE)	1 d	"
³ H-benzo[a]pyrene	Northern pike	294(E)	23 d	Balk <u>et al.</u> 1984

Footnote: NE - BCF calculated under non-equilibrium conditions
E - BCF calculated under equilibrium conditions

TABLE 2.1.22
HALF-LIFE OF PAH COMPOUNDS DETERMINED FOR FISH SPECIES

PAH	Species	Exposure Route	t 1/2	Reference
Anthracene	Bluegill Sunfish	5 h aqueous	17 h	Spacie <u>et al.</u> 1983
"	Rainbow trout	Single oral dose	7 d	Niimi & Palazo 1986
Benzo[a]pyrene	Atlantic cod yolk-sac larvae	1 d aqueous	<1 d	Solbakken <u>et al.</u> 1984
"	Bluegill Sunfish	5 h aqueous	67 h	Spacie <u>et al.</u> 1983
"	Rainbow trout	Single oral dose	<2 h	Niimi & Palazo 1986
Fluorene	Rainbow trout	Single oral dose	7 d	"
Fluoranthene	"	"	6 d	"
Naphthalene	Atlantic cod yolk-sac larvae	1 d aqueous	<1 d	" Solbakken <u>et al.</u> 1984
Phenanthrene	"	"	2 d	"
Phenanthrene	Rainbow trout	Single oral dose	9 d	Niimi & Palazo 1986

The tissue distribution of PAHs and their metabolites in fish generally reflects tissue lipid content (Solbakken et al. 1979, Whittle et al. 1977), with exceptions of gills, gall bladder and kidney (Table 2.1.23). Typically the gall bladder and bile show the highest levels of PAH and metabolites if the exposure period is long enough to allow for metabolism. Estimates of BCF for gall bladder are often an order of magnitude higher than any other tissue. This makes bile an effective means of detecting exposures of feral populations to PAHs (Krahn et al. 1984,1986). Balk et al. (1984) determined that 93 - 99% of benzo[a]pyrene in bile of northern pike (Esox lucius) was metabolized in some way; Stein et al. (1984) also found that 85-99% of B[a]P in bile of English sole (Parophrys vetulus) was present as metabolites. Muscle tissue shows low bioconcentration of all PAH examined.

Hose et al. (1981) demonstrated significant transfer of benzo[a]pyrene to eggs of marine flatfish in the ovary. The resulting B[a]P levels in eggs were sufficient to cause reduced hatch success. Southworth et al. (1979b) found that 11% of the body burden of acridine was in the ovaries of fathead minnows while testes contained roughly 1% of the body burden of acridine in males.

TABLE 2.1.23
TISSUE DISTRIBUTION OF PAH AND METABOLITES IN FISH

Chemical	Species	Tissue	Exposure Duration	Concentration		BCF	Comments/References
				Water	Tissue		
³ H-Benzo[a]pyrene	Mudsucker (<u>Gillichthys</u> <u>mirabilis</u>)	Gall bladder	96 h	1 µg/L	740 µg/100g*	740	Lee <u>et al.</u> , 1972b *Tissue concentration as µg/100 g dry weight. Parent compound.
		Liver			10	10	
		Gut			18	18	
		Gill			29	29	
		Heart			30	30	
		Flesh			2	2	
³ H-Benzo[a]pyrene	English sole (<u>Parophrys</u> <u>vetulus</u>)	Bile	10 d	*3 µg/g sediment (dry weight)	**70 ± 60 nmole/g**	14	Stein <u>et al.</u> , 1984 *Sediment dry wt concentration **tissue dry weight concentration based on total radioactivity.
		Liver			1.6 ± 0.6	0.3	
		Kidney			0.37 ± 0.3	-	
		Gill			1.0 ± 0.5	-	
		Brain			Not Detected	-	
		Blood			0.19 ± 0.17	-	
		Muscle			0.011 ± 0.005	0.001	
		Skin			0.21 ± 0.16	-	
¹⁴ C-Anthracene	Bluegill Sunfish	Gall bladder	4 h	5.8 µg/L	43,800 ng/g*	1890	Spacie <u>et al.</u> , 1983 *ng/g dry tissue weight based on radioactivity of parent compound and metabolites -estimates are not at equilibrium
		Liver			13,000	561	
		Viscera			14,900	641	
		Brain			12,900	555	
		Carcass			962	42	
¹⁴ C-Benzo[a]pyrene	Bluegill Sunfish	Gall bladder	4 h	0.7 µg/L	39,000 ng/g*	1400	-estimated BCF values: Anthracene = 900 B[a]P = 4900
		Liver			4,600	1600	
		Viscera			2,200	770	
		Brain			250	90	
		Carcass			370	130	
³ H-Benzo[a]pyrene	Northern Pike	Bile & Gall Bladder	8.5 h	75 ng/L	61.0%	82916	Balk <u>et al.</u> , 1984 *tissue level given as % of total body burden -whole body BCF = 301 -levels are parent and metabolite compounds
		Liver			12.0%	1276	
		Gills			5.0%	373	
		Intestine			4.3%	619	
		Intestine Contents			9.6%	-	
		Kidney			4.0%	-	
		Muscle			1.3%	4.6	

2.2 AQUATIC INVERTEBRATES

2.2.1 Lethal Toxicity

There are substantially more data on the acute toxicity of PAH to invertebrates than to fish, although the primary studies are limited to only thirteen compounds (Table 2.2.1). Studies which constitute secondary data are more numerous and encompass 23 compounds and/or derivatives (Table 2.2.2). Taken together, the primary and secondary data bases are useful in that they address a diversity of compounds including several examples of large molecular weight (e.g. benzo[a]pyrene, chrysene, perylene) and various degrees of side chain alkylation.

On the basis of the primary data, a considerable range of toxicity exists for the species and compounds tested. The most toxic effect observed was a 96-h LC50 of 5 μ g/L for Daphnia pulex exposed to benzo[a]pyrene (Trucco et al. 1983), while the least toxic effect observed was a 96-h LC50 of 24.5 mg/L for adult snails (Aplexa hypnorum) exposed to acenaphthene (Holcombe et al. 1983). These two studies represent a difference in toxicity by a factor of approximately 5000. Studies from the secondary database further increase this range and emphasize several factors which influence PAH toxicity to invertebrates.

2.2.1.1 Sources of Variation

As with fish, much of the observed variation in toxicity arises from bioassay logistics. PAH are volatile, a characteristic which varies inversely with molecular weight and ring number (Rossi and Neff 1978). Bioassays with invertebrates do not typically use a continuous-flow design; consequently, concentrations are not maintained unless the vessel is covered and the test is of short duration. This problem has been countered in recent work by using flow-through or static-renewal

assays. All of the primary studies listed (Table 2.2.1) are based on carefully conducted assays which attempted to minimize the potential for volatilization.

Volatilization of PAH will exaggerate the trend of increasing toxicity with increasing molecular weight because lighter compounds such as naphthalene tend to disappear quickly, and hence appear less toxic than heavier, less volatile compounds (Rossi and Neff 1978). The volatility problem also adds uncertainty to comparisons among laboratories. Lemke and Anderson (1984) commissioned standardized static toxicity assays of acenaphthene with chironomid larvae, Paratanytarsus sp. to five laboratories. Despite the use of a common source for both acenaphthene and test organisms, 48-h LC50s ranged from 0.06 to 2.0 mg/L, and two laboratories were unable to establish an LC50 because of insufficient mortality. However, each laboratory produced replicable results on a second trial. The variation in estimated LC50 apparently resulted from evaporation of acenaphthene, influenced by such factors as surface area of the test vessel and whether it was covered. With respect to other PAH, this source of uncertainty will be worst for naphthalene, the lightest and most volatile PAH, and probably accounts for the divergent naphthalene toxicity noted in Tables 2.2.1 and 2.2.2.

Photo-induced toxicity of PAH to invertebrates is also a factor influencing the range of lethal toxicity, as already noted for fish. The importance of photoinduced toxicity (e.g. increased acute lethal toxicity) is discussed in section 2.4. In the present case, it should be noted that the study conducted by Trucco et al. (1983, Table 2.2.1) involved a combination of fluorescent and natural light. It is therefore possible that a minor amount of photoinduced toxicity occurred and may account for the high toxicity noted for each of the compounds tested in their study (benz[a]anthracene, naphthalene, phenanthrene and benzo[a]pyrene). Photo-induced toxicity of PAH compounds to invertebrates are noted as secondary studies in Table 2.2.2. It should be emphasized, however, that the majority of the

TABLE 2.2.1
PRIMARY DATA OF ACUTE LETHAL TOXICITY OF PAHS TO FRESHWATER INVERTEBRATES

Chemical	Test Organism	Life Stage or Size	Method	Effect	Concentration (mg/L)	Temperature (°C)	Dissolved Oxygen (mg/L)	pH	Reference
Acenaphthene	Midge <u>Paratanytarsus</u> sp.	3rd Instar	S,M	48-h LC50	1.62	19.4-20.5	--	8.0-8.2	Lenke & Anderson 1984
	Midge <u>Paratanytarsus</u> sp.	3rd Instar	S,M	48-h LC50	1.65	19.4-20.5	--	8.0-8.2	Lenke & Anderson 1984
	Midge <u>Paratanytarsus</u> sp.	3rd Instar	S,M	48-h LC50	2.00	20.0	--	6.5-7.7	Lenke & Anderson 1984
	Midge <u>Paratanytarsus</u> sp.	3rd Instar	S,M	48-h LC50	2.09	20.0	--	6.5-7.7	Lenke & Anderson 1984
	Midge <u>Paratanytarsus</u> sp.	3rd Instar	S,M	48-h LC50	0.06	19-22	--	7.6	Lenke & Anderson 1984
	Midge <u>Paratanytarsus</u> sp.	3rd Instar	S,M	48-h LC50	0.07	19-22	--	7.6	Lenke & Anderson 1984
	Midge <u>Paratanytarsus</u> sp.	3rd Instar	S,M	48-h LC50	0.14	20-21.5	--	7.6-7.8	Lenke & Anderson 1984
	Midge <u>Paratanytarsus</u> sp.	3rd Instar	S,M	48-h LC50	0.47	20-21.5	--	7.6-7.8	Lenke & Anderson 1984
	Snail <u>Aplexa hypnorum</u>	Adult	FT,M,NA	96-h LC50	245	22.9	7.2	7.5-7.6	Holcombe <u>et al.</u> 1983
Acridine	Amphipod <u>Gammarus minus</u>	Adult	S,M,NA	48-h LC50	1.87	21-24	7.5-8.5	7.8	Millemann <u>et al.</u> 1984
	Cladoceran <u>Daphnia magna</u>	1st Instar Juvenile	SS,M,NA	96-h EC50 ^a	3.11	21	7.9-8.1	7.5-8.4	Blaylock <u>et al.</u> , 1985
	Cladoceran <u>Daphnia magna</u>	1st Instar Juvenile	S,M,NA	48-h EC50 ^a	2.05	19.5-20.5	8.0-9.0	7.8	Millemann <u>et al.</u> 1984
	Cladoceran <u>Daphnia magna</u>	1st Instar Juvenile	S,M,NA	48-h EC50 ^a	2.30	19.5-20.5	7.5-8.5	7.8	Parkhurst <u>et al.</u> 1981
	Copepod <u>Diaptomus clavipes</u>	egg to NII	SS,M,NA	85-h LC50	1.59	16	7.5-9.5	7.0-8.0	Cooney & Gehrs 1984
	Copepod <u>Diaptomus clavipes</u>	egg to NII	SS,M,NA	52-h LC50	3.44	21	7.5-9.5	7.0-8.0	Cooney & Gehrs 1984

TABLE 2.2.1 (continued)
PRIMARY DATA OF ACUTE LETHAL TOXICITY OF PAHS TO FRESHWATER INVERTEBRATES

Chemical	Test Organism	Life Stage or Size	Method	Effect	Concentration (mg/L)	Temperature (°C)	Dissolved Oxygen (mg/L)	pH	Reference
Acridine (Cont'd)	Copepod <u>Diaptomus clavipes</u>	egg to NII	SS,M,NA	36-h LC50	3.60	26	7.5-9.5	7.0-8.0	Cooney & Gehrs 1984
	Copepod <u>Diaptomus clavipes</u>	NI to CI	SS,M,NA	212-h LC50	1.55	16	7.5-9.5	7.0-8.0	Cooney & Gehrs 1984
	Copepod <u>Diaptomus clavipes</u>	NI to CI	SS,M,NA	84-h LC50	1.75	21	7.5-9.5	7.0-8.0	Cooney & Gehrs 1984
	Copepod <u>Diaptomus clavipes</u>	NI to CI	SS,M,NA	142-h LC50	1.18	26	7.5-9.5	7.0-8.0	Cooney & Gehrs 1984
	Midge <u>Chironomus tentans</u>	4th Instar Larva	S,M,NA	48-h LC50	1.86	23-26	7.0-8.0	7.8	Millemann <u>et al.</u> 1984
	Snail <u>Physa gyrina</u>	Adult	S,M,NA	48-h LC50	11.0	19.5-20.5	8.0-9.0	7.8	Millemann <u>et al.</u> 1984
Anthracene	Cladoceran <u>Daphnia pulex</u>	1st Instar Juvenile	S,M,NA	48-h EC50 ^a	0.75	20.0	8.0-9.0	--	Smith <u>et al.</u> 1988
Benz[a]-anthracene	Cladoceran <u>Daphnia pulex</u>	1.9-2.1 mm	S,M,A	96-h LC50	0.010	13-17	6.5-7.5	7.5	Trucco <u>et al.</u> 1983
Methyl-anthracene	Cladoceran <u>Daphnia pulex</u>	1st Instar Juvenile	S,N,NA	48-h EC50 ^a	0.096	20.0	8.0-9.0	--	Smith <u>et al.</u> 1988
9-Methoxy-anthracene	Cladoceran <u>Daphnia pulex</u>	1st Instar Juvenile	S,N,NA	48-h EC50 ^a	0.40	20.0	8.0-9.0	--	Smith <u>et al.</u> 1988
Fluorene	Amphipod <u>Gammarus pseudolimnaeus</u>	3-4 mm	S,M,NA	96-h LC50	0.60	17	8.0-9.0	7.2-7.4	Finger <u>et al.</u> 1985
	Cladoceran <u>Daphnia magna</u>	1st Instar Juvenile	S,M,NA	48-h EC50 ^a	0.43	17	8.0-9.0	7.2-7.4	Finger <u>et al.</u> 1985
	Cladoceran <u>Daphnia pulex</u>	1st Instar Juvenile	S,N,NA	48-h EC50 ^a	0.21	20.0	8.0-9.0	7.7-7.8	Smith <u>et al.</u> 1988
	Mayfly <u>Hexagenia bilineata</u>	5-6 mm	S,M,NA	120-h LC50	5.8	22	8.0-9.0	7.2-7.4	Finger <u>et al.</u> 1985
	Midge <u>Chironomus riparius</u>	3rd Instar Larva	S,M,NA	48-h EC50	2.35	22	8.0-9.0	7.2-7.4	Finger <u>et al.</u> 1985

TABLE 2.2.1 (continued)
PRIMARY DATA OF ACUTE LETHAL TOXICITY OF PAHS TO FRESHWATER INVERTEBRATES

Chemical	Test Organism	Life Stage or Size	Method Effect	Concentration (mg/L)	Temperature (°C)	Dissolved Oxygen (mg/L)	pH	Reference
Fluorene (Cont'd)	Snail <u>Mudalia potosensis</u>	Adult	S,M,NA 96-h LC50	5.60	17	8.0-9.0	7.2-7.4	Finger <u>et al.</u> 1985
Naphthalene	Amphipod <u>Gammarus minus</u>	Adult	S,M,NA 48-h LC50	3.93	21-24	7.5-8.5	7.8	Millemann <u>et al.</u> 1984
	Cladoceran <u>Daphnia magna</u>	1st Instar Juvenile	S,M,NA 48-h EC50 ^a	2.16	19.5-20.5	8.0-9.0	7.8	Millemann <u>et al.</u> 1984
	Cladoceran <u>Daphnia pulex</u>	1st Instar Juvenile	S,N,NA 48-h EC50 ^a	4.66	20.0	8.0-9.0	--	Smith <u>et al.</u> 1988
	Cladoceran <u>Daphnia pulex</u>	1.9-2.1 mm	S,M,A 96-h LC50	1.0	13-17	6.5-7.5	7.5	Trucco <u>et al.</u> 1983
	Midge <u>Chironomus tentans</u>	4th Instar Larva	S,M,NA 48-h LC50	2.81	23-26	7.0-8.0	7.8	Millemann <u>et al.</u> 1984
	Snail <u>Physa gyrina</u>	Adult	S,M,NA 48-h LC50	5.02	19.5-20.5	8.0-9.0	7.8	Millemann <u>et al.</u> 1984
1,3-Dimethyl-naphthalene	Cladoceran <u>Daphnia pulex</u>	1st Instar Juvenile	S,N,NA 48-h EC50 ^a	0.77	20.0	8.0-9.0	--	Smith <u>et al.</u> 1988
	Cladoceran <u>Daphnia pulex</u>	1st Instar Juvenile	S,N,NA 48-h EC50 ^a	1.28	17	8.0-9.0	--	Passino & Smith 1987
2,6-Dimethyl-naphthalene	Cladoceran <u>Daphnia pulex</u>	1st Instar Juvenile	S,N,NA 48-h EC50 ^a	0.19	20.0	8.0-9.0	--	Smith <u>et al.</u> 1988
2-Methoxy-naphthalene	Cladoceran <u>Daphnia pulex</u>	1st Instar Juvenile	S,N,NA 48-h EC50 ^a	4.04	17	8.0-9.0	--	Passino & Smith 1987
Phenanthrene	Amphipod <u>Gammarus minus</u>	Adult	S,M,NA 48-h LC50	0.46	21-24	7.5-8.5	7.8	Millemann <u>et al.</u> 1984
	Cladoceran <u>Daphnia magna</u>	1st Instar Juvenile	S,M,NA 48-h EC50 ^a	0.70	19.5-20.5	8.0-9.0	7.8	Millemann <u>et al.</u> 1984
	Cladoceran <u>Daphnia pulex</u>	1st Instar Juvenile	S,N,NA 48-h EC50 ^a	0.734	17	8.0-9.0	7.7-7.8	Passino & Smith 1987
	Cladoceran <u>Daphnia pulex</u>	1st Instar Juvenile	S,N,NA 48-h EC50 ^a	0.35	20	8.0-9.0	--	Smith <u>et al.</u> 1988
	Cladoceran <u>Daphnia pulex</u>	1.9-2.1 mm	S,M,A 96-h LC50	0.10	13-17	6.5-7.5	7.5	Trucco <u>et al.</u> 1983

TABLE 2.2.1 (continued)
PRIMARY DATA OF ACUTE LETHAL TOXICITY OF PAHS TO FRESHWATER INVERTEBRATES

Chemical	Test Organism	Life Stage or Size	Method	Effect	Concentration (mg/L)	Temperature (°C)	Dissolved Oxygen (mg/L)	pH	Reference
Phenanthrene (Cont'd)	Midge <u>Chironomus tentans</u>	4th Instar Larva	S,M,NA	48-h LC50	0.49	23-26	7.0-8.0	7.8	Millemann <u>et al.</u> 1984
Benzo[a]-pyrene	Cladoceran <u>Daphnia pulex</u>	1.9-2.1 mm	S,M,A	96-h LC50	0.005	13-17	6.5-7.5	7.5	Trucco <u>et al.</u> 1983

Footnotes: a - endpoint of bioassay was immobilization

S - static bioassay

SS - semi static bioassay

FT - flow through bioassay

M - measured exposure concentrations

N - nominal exposure concentrations

A - aerated exposure vessel

NA - no aeration in exposure vessel

TABLE 2.2.2
SECONDARY DATA OF ACUTE LETHAL TOXICITY OF PAHS TO FRESHWATER INVERTEBRATES

Chemical	Test Organism	Life Stage or Size	Method	Effect	Concentration (mg/L)	Temperature (°C)	Dissolved Oxygen (mg/L)	pH	Reference
Acenaphthene	Cladoceran <u>Daphnia magna</u>	1st Instar Juvenile	S,N,NA	48-h LC50	41.0	22	--	8.0	LeBlanc 1980
Acridine	Cladoceran <u>Daphnia magna</u>	1st Instar Juvenile	SS,M,NA	UV-LT50	0.90/0.440 ^C	23	7.0	7.5	Newsted & Giesy 1987
	Cladoceran <u>Daphnia pulex</u>	Adult (ca. 0.6mg)	S,M	24-h LC50	2.92	21	5-8	7.5-7.9	Southworth <u>et al.</u> 1978b
Benz[a]-acridine	Cladoceran <u>Daphnia pulex</u>	Adult (ca. 0.6mg)	S,M	24-h LC50	0.449	21	5-8	7.5-7.9	Southworth <u>et al.</u> 1978b
Anthracene	Cladoceran <u>Daphnia magna</u>	1st Instar Juvenile	SS,M,NA	UV-LT50	4.98/0.015 ^C	23	7.0	7.5	Newsted & Giesy 1987
	Cladoceran <u>Daphnia magna</u>	Adult	S,M	1-h LC50 ^b	0.02	--	--	--	Kagan <u>et al.</u> 1985
	Dipteran <u>Aedes aegypti</u>	1st Instar Larva	S,M	1-h LC50 ^b	0.15	--	--	--	Kagan <u>et al.</u> 1985
Benz[a]-anthracene	Cladoceran <u>Daphnia magna</u>	1st Instar Juvenile	SS,M,NA	UV-LT50	12.51/0.002 ^C	23	7.0	7.5	Newsted & Giesy 1987
Benz[b]-anthracene	Cladoceran <u>Daphnia magna</u>	1st Instar Juvenile	SS,M,NA	UV-LT50	16.43/0.001 ^C	23	7.0	7.5	Newsted & Giesy 1987
Dibenz[ah]-anthracene	Cladoceran <u>Daphnia magna</u>	1st Instar Juvenile	SS,M,NA	UV-LT50	3.08/0.004 ^C	23	7.0	7.5	Newsted & Giesy 1987
Chrysene	Cladoceran <u>Daphnia magna</u>	1st Instar Juvenile	SS,M,NA	UV-LT50	23.98/0.002 ^C	23	7.0	7.5	Newsted & Giesy 1987

TABLE 2.2.2 (continued)
SECONDARY DATA OF ACUTE LETHAL TOXICITY OF PAHs TO FRESHWATER INVERTEBRATES

Chemical	Test Organism	Life Stage or Size	Method	Effect	Concentration (mg/L)	Temperature (°C)	Dissolved Oxygen (mg/L)	pH	Reference
Fluoranthene	Cladoceran <i>Daphnia magna</i>	1st Instar Juvenile	SS,M,NA	UV-LT50	10.8/0.009 ^C	23	7.0	7.5	Newsted & Giesy 1987
	Cladoceran <i>Daphnia magna</i>	1st Instar Juvenile	S,N,NA	48-h LC50	320.0	22	--	8.0	LeBlanc 1980
	Cladoceran <i>Daphnia magna</i>	Adult	S,M	1-h LC50 ^b	0.004	--	--	--	Kagan <i>et al.</i> 1985
	Dipteran <i>Aedes aegypti</i>	1st Instar Larva	S,M	1-h LC50 ^b	0.12	--	--	--	Kagan <i>et al.</i> 1985
1H-Benzo[k]-fluoranthene	Cladoceran <i>Daphnia magna</i>	1st Instar Juvenile	SS,M,NA	UV-LT50	13.0/0.001 ^C	23	7.0	7.5	Newsted & Giesy 1987
1H-Benzo[a]-fluorene	Cladoceran <i>Daphnia magna</i>	1st Instar Juvenile	SS,M,NA	UV-LT50	22.95/0.05 ^C	23	7.0	7.5	Newsted & Giesy 1987
1H-Benzo[b]-fluorene	Cladoceran <i>Daphnia magna</i>	1st Instar Juvenile	SS,M,NA	UV-LT50	22.40/0.002 ^C	23	7.0	7.5	Newsted & Giesy 1987
Naphthalene	Cladoceran <i>Daphnia magna</i>	1st Instar Juvenile	S,N,NA	48-h LC50	8.60	22	--	8.0	LeBlanc 1980
	Copepod <i>Eurytemora affinis</i>	Adult	S,M,NA ^a	24-h LC50	3.80	15	--	--	Ott <i>et al.</i> 1978
1-chloro-naphthalene	Cladoceran <i>Daphnia magna</i>	1st Instar Juvenile	S,N,NA	48-h LC50	1.60	22	--	8.0	LeBlanc 1980
Octachloro-naphthalene	Cladoceran <i>Daphnia magna</i>	1st Instar Juvenile	S,N,NA	48-h LC50	>530.0	22	--	8.0	LeBlanc 1980
2-methyl-naphthalene	Copepod <i>Eurytemora affinis</i>	Adult	S,M,NA ^a	24-h LC50	1.50	15	--	--	Ott <i>et al.</i> 1978
2,6-dimethyl-naphthalene	Copepod <i>Eurytemora affinis</i>	Adult	S,M,NA ^a	24-h LC50	0.85	15	--	--	Ott <i>et al.</i> 1978
2,3,5-tri-methylnaphthalene	Copepod <i>Eurytemora affinis</i>	Adult	S,M,NA ^a	24-h LC50	0.32	15	--	--	Ott <i>et al.</i> 1978
Perylene	Cladoceran <i>Daphnia magna</i>	1st Instar Juvenile	SS,M,NA	UV-LT50	18.33/0.001 ^C	23	7.0	7.5	Newsted & Giesy 1987

TABLE 2.2.2 (continued)
SECONDARY DATA OF ACUTE LETHAL TOXICITY OF PAHS TO FRESHWATER INVERTEBRATES

Chemical	Test Organism	Life Stage or Size	Method	Effect	Concentration (mg/L)	Temperature (°C)	Dissolved Oxygen (mg/L)	pH	Reference
Benzo[ghi]-perylene	Cladoceran <u>Daphnia magna</u>	1st Instar Juvenile	SS,M,NA	UV-LT50	13.82/0.0002 ^C	23	7.0	7.5	Newsted & Giesy 1987
Pyrene	Cladoceran <u>Daphnia magna</u>	1st Instar Juvenile	SS,M,NA	UV-LT50	3.47/0.006 ^C	23	7.0	7.5	Newsted & Giesy 1987
	Cladoceran <u>Daphnia magna</u>	Adult	S,M	1-h LC50 ^b	0.004	--	--	--	Kagan <u>et al.</u> 1985
	Dipteran <u>Aedes aegypti</u>	1st Instar Larva	S,M	1-h LC50 ^b	0.02	--	--	--	Kagan <u>et al.</u> 1985
Benzo[a]-pyrene	Cladoceran <u>Daphnia magna</u>	1st Instar Juvenile	SS,M,NA	UV-LT50	4.44/0.002 ^C	23	7.0	7.5	Newsted & Giesy 1987
Benzo[e]-pyrene	Cladoceran <u>Daphnia magna</u>	1st Instar Juvenile	SS,M,NA	UV-LT50	15.26/0.001 ^C	23	7.0	7.5	Newsted & Giesy 1987

Footnotes: a - tested under estuarine conditions (20% seawater)
b - phototoxicity bioassay, mortalities quantified over 1 hour of U.V. exposure
c - phototoxicity bioassay, time to 50% mortality (hr)/exposure concentration (mg/L)
S - static bioassay
SS - semi static bioassay
M - measured exposure concentrations
N - nominal exposure concentrations
NA - no aeration in exposure vessel

compounds subject to photoinduced toxicity, were determined through carefully controlled and reliable tests (Newsted and Giesy 1987). They have been classified as secondary data because of the use of a nonstandard response variable which describes the time required to yield 50% mortality under a defined PAH concentration and ultra-violet light exposure (UV-LT50). Presently, however, the use of the UV-LT50 endpoint seems unavoidable because of the rapid rate of toxicity (usually within 15 h) at extremely low concentrations (1.0 and 10.0 $\mu\text{g/L}$) and the dependency on ultra-violet light intensity. These "secondary" data are therefore regarded as quality data.

Real variation among species is undoubtedly the cause of part of the observed range of sensitivity to PAH. In particular, the resistance of snails is probably a consequence of this group's adaptation to slow currents, low oxygen levels and muddy substrates. It may be significant that burrowing mayflies (Hexagenia sp.) and the mosquito fish (Gambusia affinis) which also exhibit extraordinary resistance to PAH toxicity, are similarly adapted. Differences in the ability to metabolize and depurate PAH may also contribute to this variation.

There is also limited evidence that acute toxicity of PAH varies with temperature (Smith et al. 1988). Their results when compared to the data of Passino and Smith (1987), demonstrate an increase in the toxicity of both 1,3-dimethylnaphthalene and phenanthrene to Daphnia pulex over a temperature increase from 17° to 20°C. Cooney and Gehrs (1984) have studied temperature effects in more detail using the freshwater calanoid copepod, Diaptomus clavipes, and acridine. They determined LC50s of immature copepods during either the egg-to-second-naupliar stage, or the later first-nauplius-to-first-copepod stage. Their results show that toxicity declines (LC50 increasing) with increasing temperature for eggs and young nauplii, but the trend for older organisms is interrupted by the sharply lower LC50 at 26°C (Table 2.2.1). The results are complicated because development time (and hence exposure to acridine) decreased as temperature increased.

These results contradict those of Smith et al. (1988) and indicate that more work is required before a full appreciation of temperature effects on PAH toxicity to invertebrates can be obtained.

2.2.1.2 Trends in Toxicity

Several trends in acute toxicity are apparent in the invertebrate data. For the purposes of ranking, LC50's and EC50's from the primary data set were converted to a micromolar basis. Only studies in which Daphnia magna or Daphnia pulex were the test organisms used; these were invariably the most sensitive invertebrates and provided the greatest consistency. An exception is the case of acenaphthene, where only the midge, Paratanytarsus sp. was tested. Whenever possible, only 48-h LC50's or EC50's (immobilization) were considered. In the case of benz[a]anthracene and benzo[a]pyrene, however, only 96-h LC50s were available. The ranking of compounds in order of increasing toxicity is summarized in Table 2.2.3.

Ranking of the toxicity data indicate the addition of alkyl side chains increases toxicity to varying extents. For example, the addition of a methoxy group caused only minor increases in toxicity for naphthalene and anthracene, while methylation of naphthalene increased toxicity by as much as 25 times (Smith et al. 1988).

The position of side chain alkylation is also an important feature. In the case of naphthalene, the 2,6-dimethyl derivative was approximately five times more toxic than the 1,3-dimethyl derivative. Increased toxicity as a result of methylation was also reported for naphthalene derivatives by Ott et al. (1978) with the copepod, Eurytemora affinis (Table 2.2.2). The effect, however, was less in magnitude than that reported by Smith et al. (1988), with the addition of each methyl group resulting in an apparent doubling of toxicity over a 24 h exposure (Figure 2.2.1).

TABLE 2.2.3
RANKING OF PRIMARY LETHAL TOXICITY DATA FOR INVERTEBRATES
SEE TEXT FOR DETAILS OF RANKING PROCEDURE

Chemical	MW	Test Organism ^a	Effect	Mean Conc. (μ M)
Naphthalene	128.0	Daphnia sp.	48-h EC50 ^b	26.640
2-Methoxynaphthalene	158.2	D. pulex	48-h EC50	25.540
Acridine	179.2	Daphnia sp.	48-h EC50	12.137
Acenaphthene	154.0	Midge	48-h LC50	6.574
1,3-Dimethylnaphthalene	156.0	D. pulex	48-h EC50	4.969
Anthracene	178.2	D. pulex	48-h EC50	4.209
Phenanthrene	178.2	Daphnia sp.	48-h EC50	3.337
Fluorene	166.2	Daphnia sp.	48-h EC50	1.925
9-Methoxyanthracene	208.2	D. pulex	48-h EC50	1.920
2,6-Dimethylnaphthalene	156.0	D. pulex	48-h EC50	0.920
Methylanthracene	192.0	D. pulex	48-h EC50	0.499
Benz[a]anthracene	228.0	D. pulex	96-h LC50	0.044
Benzo[a]pyrene	252.3	D. pulex	96-h LC50	0.020

Footnotes: a - Cases in which data for D. magna and D. pulex were combined are noted as Daphnia sp.

b - EC50 pertains to immobilization

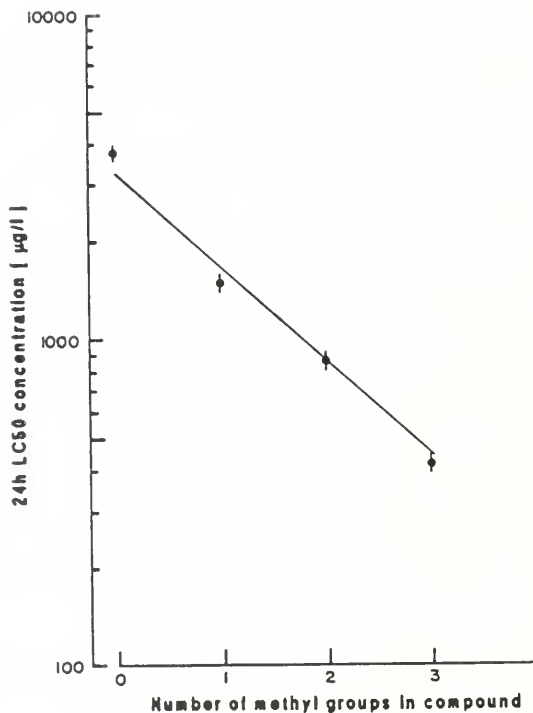


Figure 2.2.1 Relationship between 24-h LC50 concentration determined for adult female Eurytemora affinis and number of methyl groups in the naphthalene to which the animals were exposed. (Log Y = $-0.24x + 3.56$; $r = -0.99$). LC50 levels, with 95% confidence limits. In µg/L: $N = 3798 \pm 11.2$; $DMN = 852 \pm 17.7$; $TMN = 316 \pm 20.9$.

(From Ott et al. 1978)

Toxicity also increases with increasing degree of ring structure, and therefore molecular weight, although the relationship exhibits some exceptions. Trucco et al. (1983) reported an exponential increase in toxicity (96-h LC50's, *D. pulex*) with respect to molecular weight for benzene, phenanthrene, benz[a]anthracene and benzo[a]pyrene. The consolidated data of Table 2.2.3 also demonstrate the least toxic compounds are in general the simpler, unsubstituted PAH (e.g. naphthalene, acenaphthene), while the more complex aromatics (e.g. benz[a]anthracene, benzo[a]pyrene) are more toxic.

The ranking given here reflects the conventional acute toxicity of these compounds. The photoinduced toxicity of many PAH presents a much higher degree of potency. Many, but not all of the PAH, could be considered equipotent in terms of photoinduced toxicity, since normalized UV-LT50's are typically less than 18 h for aqueous concentrations in the range of 0.010 to 0.001 mg/L. In addition, tests with both conventional and photoinduced toxicity suggest that the compounds which are the most acutely toxic may also be the most carcinogenic or mutagenic (e.g. benzo[a]pyrene, dibenz[ah]anthracene). Therefore, two entirely different forms of toxicity emphasize the hazards associated with the more complex PAH.

2.2.1.3 Chronic Lethalities

There are few data on the long-term lethal toxicity of PAH to invertebrates. Bendugo et al. (1977) observed 30% mortality in the marine copepod *Eurytemora affinis* after a 10-d exposure to 0.05 mg/L naphthalene. This is 75 times lower than the 24-h LC50 reported by Ott et al. (1978, Table 2.2.2). Much of this difference may be due to differences in the experimental procedures, since the figure of Ott et al. (1978) is based on static data.

Continuous exposure of Daphnia magna to fluorene (≤ 0.125 mg/L) in a 21-day life-cycle test was reported by Finger et al. (1985). Adult mortalities were not dose dependent and did not exceed 10%. In the same study, a 30-day exposure of the midge, Chironomus riparius, to 0.6 mg/L resulted in only 50% emergence. This concentration represents 26% of their estimated 48-h EC50 for immobilization (Table 2.2.1).

Chronic exposure of freshwater copepods, (Diaptomus clavipes), to acridine (1.8 mg/L), from the first naupliar stage to the first copepodid stage, resulted in an LT50 of 16 days at a temperature of 16°C (Cooney and Gehrs 1984).

2.2.1.4 Mixture Toxicity

Studies of the toxicity of PAH mixtures to freshwater invertebrates are also limited, and seldom report adequate data on mixture composition. Eastmond et al. (1983) studied the lethal toxicity of the whole fraction of a coal liquefaction product using an acetone carrier to facilitate exposure to the less soluble components (Table 2.2.4). Various forms of PAH (unidentified) accounted for approximately 90% of the carbon composition. The results of acute lethal bioassays indicated the cladoceran, D. magna, to be most sensitive, while the snail, Physa sp., and clam, U. lamsilis, were least sensitive. The range of toxicity, 0.52 to greater than 5.0 mg/L, is representative of the toxicity for many individual compounds. Because of the lack of controlled mixture toxicity studies of PAH to invertebrates, it is not possible to clearly define the interactive effects of the compounds with respect to acute lethal effects. The observation that complex mixtures do not evoke notably increased toxicity in relation to toxicity of pure compounds, however, suggests that a synergistic model is unlikely.

TABLE 2.2.4
SECONDARY DATA OF ACUTE LETHAL TOXICITY OF PAH MIXTURES TO FRESHWATER INVERTEBRATES

Industrial Mixture	Composition (mg/L)	Test Organism	Life Stage or Size	Method	Effect	Concentration (mg/L)	pH	Comments	Reference
Solvent Refined Coal-II Heavy Distillate (Coal Liquefaction Product)	5-6% Aliphatic 59-69% PAH + PASH ^a 17-21% PANH ^b 9-14% HPAH ^c	Amphipod <u>Hyaletella azteca</u>	Adult	FT,M	96-h LC50	0.74	7.4	-Log k_{ow} =3.53 -Acetone carrier used to deliver toxicant	Eastmond <u>et al.</u> 1983
		Cladoceran <u>Daphnia magna</u>		S,M	48-h LC50	0.52	7.4		
		Clam <u>Urio lamellis</u>	Adult	FT,M	96-h LC50	>5.0	7.4		
		Crayfish <u>Orconectes virillis</u>	Adult	FT,M	96-h LC50	1.66	7.4		
		Snail <u>Physa</u> spp.	Adult	FT,M	96-h LC50	>5.0	7.4		

Footnotes: a - polycyclic aromatic sulfur heterocycles
b - polycyclic aromatic nitrogen heterocycles
c - hydroxylated aromatic hydrocarbons
FT - flow through bioassay
S - static bioassay
M - measured exposure concentrations

2.2.2 Sublethal Toxicity

In general, the available literature on sublethal acute and subacute toxicity of PAH to aquatic invertebrates is much weaker than that available for lethal toxicity. In addition, freshwater invertebrates are poorly represented, hence, the necessity to consider marine data in some cases. Because the number of PAH are also few, studies which address sources of PAH (e.g. water soluble fractions of crude oil) have been considered. Accordingly, the majority of information addressed in this section should be regarded as secondary data from a regulatory perspective. Several good quality studies address chronic effects (e.g. growth, reproduction), although the number of chemicals addressed is very limited.

2.2.2.1 Acute Effects

2.2.2.1.1 Effects on membranes

PAH are lipophilic molecules which interact with lipoprotein membranes by affecting both the membrane structure and its function. Under normal conditions, lysosomal membranes are permeable to small molecules, so that disruption of normal status can be evaluated on the penetrability of an enzyme-based substrate and expressed in terms of latency to enzymatic activity.

A decrease in the lysosomal latency period of the digestive gland of the marine snail, Littorina littorea, was observed after 3 days of exposure to phenanthrene (Moore et al. 1985). A similar decrease in lysosomal membrane stability was observed in mussel, Mytilus edulis, exposed to phenanthrene and anthracene (Nott et al. 1985). The same response occurred when anthracene was injected directly into the posterior adductor muscle of the blue mussel (Moore et al. 1978).

Lysosomal stability declined linearly with increasing anthracene concentrations, while a sigmoidal relationship was observed with phenanthrene. This suggests the presence of a threshold concentration at the site of action (Moore and Farrar 1985).

This dose dependent lysosomal destabilization may result in the release of hydrolases into the cytoplasm of affected cells with accompanying evidence of cytolysis in the anthracene injected mussels. Although the destabilizing effects lasted for 96 hours, complete recovery was achieved within 168 hours after injection (Moore et al. 1978). Marine snails (L. littorea) exposed to phenanthrene at 400 $\mu\text{g/L}$ required twelve days in clean seawater for recovery of lysosomal membrane stability. A similar exposure of mussels did not allow recovery and consequently delayed injury can be anticipated in mussels exposed to PAH (Moore et al. 1985).

Simultaneous dosing of mussels with phenanthrene (100 $\mu\text{g/L}$) and copper (20 $\mu\text{g/L}$) produced an interactive effect on lysosomal membrane stability. The greatest membrane instability was observed after 3 days recovery in clean water (latency times 60%, 20% and 8% of controls for copper, phenanthrene and the mixture, respectively). Even after 12 days recovery, latency was sharply reduced in the phenanthrene (33%) and mixture (13%) treatments (Moore et al. 1984).

The exact mechanism(s) causing cell membrane damage has yet to be conclusively determined, although it has been suggested that lipid peroxidation is a factor, and this is consistent with free radical formation through PAH metabolism.

Phenanthrene also affects the morphology of mussel digestive cells, and significantly increases the smooth endoplasmic reticulum (SER) (Nott et al. 1985). The significance of these observations is still uncertain, although they are believed to be related to the increased cytochrome P-450 system activity observed during PAH exposure. Unlike phenanthrene, anthracene exposure did not elicit these morphological changes.

2.2.2.1.2 Behavior

There have been few studies of PAH effects on invertebrate behavior. Injections of 0.2 and 1.0 μg benzo[a]pyrene directly into the visceral sac of freshwater mussels (Dreissena polymorpha, Anodonta cygnea, Unio pictorum and U. tumidus), resulted in a significant decrease in filtration rate (Neff 1979). It was suggested that benzo[a]pyrene depressed ciliary activity or possibly the entire metabolism of the mussels; this is consistent with the narcotizing effect of low-level PAH intoxication sometimes observed, but the possibility of a behavioral reaction to the injection itself cannot be excluded.

Exposure to a crude oil dispersal has also elicited behavior reactions (Wong et al. 1983). Gravid adult Daphnia pulex were exposed to a nominal concentration of 50 $\mu\text{L/L}$ of a Norman crude oil emulsion (15-20% aromatic content). Immediately after the addition of oil, the frequencies of thoracic appendage movements, the mandibular beat and swallowing frequency were depressed. There was also a concomitant increase in rate of food rejection by the post abdomen and the labrum. Because the affected movements are so closely associated with feeding, these observations are significant in terms of total food consumption and bioenergetics. Further, the responses were attributed to the components of crude oil since a paraffin emulsion failed to produce the same results.

A significant reduction in the swimming ability of Daphnia sp. was also observed after 24 hours of exposure to a nominal concentration of 50 $\mu\text{L/L}$ of a Norman Well crude oil emulsion. Partial recovery was however observed 24 hours after transfer to clean water (Wong et al. 1981). It was suggested that the immobilization of Daphnia induced by crude oil might result in an increased susceptibility to predators (Engelhardt et al. 1981a).

2.2.2.2 Subacute Effects

2.2.2.2.1 Genotoxic and Teratogenic Effects in Aquatic Invertebrates

Work on field collections of freshwater invertebrates exposed to PAH is essentially non-existent. No laboratory work involving freshwater organisms was evident, although several studies are available involving marine organisms. Al-Sabti and Kurelec (1985) found an increase in chromosomal aberrations in wild mussels (Mytilus galloprovincialis) collected from areas receiving a variety of waste types. Laboratory exposure of the organisms to B[a]P (1, 5, 10 $\mu\text{g/L}$) induced an increase in aberrations in a dose-response fashion (3-fold, 7-fold, and 16-fold, respectively).

The ^{32}P post-labeling of DNA adducts as described earlier (section 2.1.2.2.2) has recently been applied to a marine bivalve Mytilus galloprovincialis (Kurelec et al. 1987). Digestive gland homogenates demonstrated in vitro DNA adducts when exposed to 2-amino fluorene but not benzo[a]pyrene, suggest a low capacity of Mytilus MFO for B[a]P metabolism.

Exposure of marine amphipods (Rhepoxynius abronius and Eohaustorius washingtonianus) to contaminated sediments spiked with ^3H -B[a]P resulted in detection of covalent binding of B[a]P derivatives to protein macromolecules (Reichert et al. 1985). Species differences indicated R. abronius generated almost double the number of covalently

bound molecules than E. washingtonianus. More importantly, however, both species demonstrated formation of the B[a]P-7,8-diol and B[a]P-9,10-diol, indicating the potential for carcinogenicity from these proximate carcinogens.

The most comprehensive studies of genotoxic effects of PAH to invertebrates involves work with sea urchins. The extensive use of these species is associated with their applicability to laboratory

studies and their fast developmental rate. Sea urchin development progresses from a single-celled egg to a 3000-cell pluteal larval stage in 48 h which facilitates rapid experimental examination for cell multiplication, differentiation and organization (de Angelis and Giordana, 1974). Diagnostic tests have been recently defined and are capable of detecting dose-dependent responses to both the egg and parental exposures to B[a]P (Hose 1985). Threshold responses vary from 0.5 to 1.0 $\mu\text{g/L}$ B[a]P (waterborne) for egg exposures and 20 to 100 mg/kg (injection) for parental exposure (*Paracentrotus purpuratus*) (Hose 1985). Responses examined included the frequency of abnormal gastrulae, mitotic figures, chromosomal aberrations, micronucleated cells and cytologic abnormalities.

Other laboratory exposures using various species of sea urchins have examined B[a]P (Ceas 1974), B[a]P and 7,12-dimethylbenz[a]-anthracene (de Angelis and Gioranda 1974), and the PAH fraction of tobacco smoke condensate (Bresch et al. 1972). Sea urchin testing offers a great deal of potential for laboratory examination of PAH impacts on invertebrates, but tests involving freshwater organisms require development. Since PAH generally requires metabolic transformation to evoke carcinogenicity, the low availability of a metabolizing system required for PAH metabolism in invertebrates, especially in the sea urchin egg exposures, likely explains the absence of laboratory incidences of PAH-induced carcinogenesis.

2.2.2.2.2 Biochemical and Physiological Effects

Biochemical

There is indirect evidence of effects on various enzyme systems; most of this work deals with organisms exposed to petroleum (water-soluble fractions) or derivatives. Widdows et al. (1982) exposed mussels to North Sea oil (30 $\mu\text{g/L}$) for 180 days, and observed significant increases in specific activities of phosphofructokinase,

phosphoenolpyruvate carboxykinase, and glucose-6-phosphate dehydrogenase in the gill and kidney; and NADP isocitrate dehydrogenase in the posterior adductor muscle. Since each of these enzymes is directly involved in the glycolytic chain suggest an elevated rate of glucose utilization and hence increased maintenance energy needs (Widdows et al. 1982). This is consistent with the elevated rate of oxygen consumption and reduced "scope for growth" exhibited by these animals. North Sea oil hydrocarbons also significantly reduced food absorption efficiency, and increased ammonia release (an additional energy loss) causing a reduction in the net energy intake of dosed mussels was reduced.

Various studies have addressed the effects of oil spills as a source of PAH exposure. Chambers et al. (1978) exposed adult brown shrimp (Penaeus sp.) to various concentrations of crude oil for 24 h and found the oils were acutely lethal in the 15-25 mg/L range. Empire Mix crude oil had no effect on any measured enzymes. In contrast, Saudi Arabian crude oil, which is richer in sulphur and polyaromatics, had marked and widespread effects on hepato-pancreatic enzymes. Evidently, exposure to high crude oil concentrations can cause in the short term dramatic physiological changes, at least in the short term. However, as in most study involving oil, the complexity of the mixtures make it impossible to measure the effect due to the PAH component.

Naphthalene exposure of mysid shrimp (Neomysis americana) induced a dose dependent modification of oxygen consumption as monitored by oxygen uptake from the water. Oxygen consumption was slightly decreased under low naphthalene concentrations (0.088 mg/L), but strongly stimulated at higher concentrations (1.11 mg/L). This increased oxygen uptake again suggests an increase in maintenance energy requirements as reflected through the standard metabolic rate. The physical disruption of the gill integument, caused by PAH, may also lead to leakage through the membrane and result in an elevated cost of osmoregulation associated with ATPase activity and tissue repair (Smith and Hargreaves 1984). However, the high exposure concentration is close to the acutely lethal range, and only the lower dose approaches environmentally realistic concentrations.

Conversely, mussels (M. californianus), exposed to a saturated solution of benzo[a]pyrene, experienced an insignificant decrease in metabolic rate (Sabourin and Tullis 1981). However, the results of this study are of limited value since the exposure concentrations were not measured.

In another investigation, mussels exposed to the water-soluble fraction (WSF) of North Sea oil (30-36 μg total hydrocarbon/L) displayed a significantly (30%) elevated rate of oxygen consumption within 7 days of exposure. This response was maintained throughout the subsequent 5-month exposure period. It was suggested that this increased rate of oxygen consumption was associated with PAH-induced biochemical changes in key metabolic enzyme (e.g. increased glucose-6-phosphate dehydrogenase) or utilization of oxygen by the mixed function oxidase (MFO) system (Widdows et al. 1982). It is noteworthy that this WSF was a complex mixture of PAH and other organic contaminants, therefore effects cannot be specifically associated with PAH.

Conversely, in another study where mussels were exposed to phenanthrene (100 $\mu\text{g/L}$) (Moore et al. 1984), no change in oxygen consumption rate was reported. Brown shrimp (Penaeus sp.), exposed to an unanalyzed Empire mix and Saudi Arabian crude oil sample, displayed changes in enzymatic patterns that suggested an increased anaerobic energy metabolism, although oxygen consumption was not monitored in this study (Chambers et al. 1978).

Mussels exposed alternately to WSF and/or algal food ceased filtering during the contaminated water exposure period. Filtration resumed when food was introduced in the aquarium. These changes could be regarded as adaptative and may provide the mussels with a means of tolerating the hydrocarbons. The reduced ventilation rate is consistent with a reduction in oxygen consumption as a consequence of exposure to an extraneous irritant. This response was well documented in mussels exposed to intertidal conditions of lower oxygen tension (Widdows et al. 1982).

In cases where depressed oxygen consumption is observed, it is usually attributed to narcosis or reduced activity in response to the toxicant (Smith and Hargreaves 1984), while elevated respiration probably reflects the metabolic cost of detoxifying. The latter, is usually observed when invertebrates are exposed to hydrocarbon mixtures such as oils and may persist for days or months as long as the toxicant is present (Widdows et al. 1982). The elevated rate of oxygen consumption is probably too small to be of consequence in itself. However, it indicates that organisms living in PAH-contaminated waters must direct a portion of their energy budget toward detoxification which compromises that available for growth, reproduction and defense. In the long-term, reductions in abundance are possible (Smith and Hargreaves 1984, Neff 1979).

The utility of respiration as an index of general stress response is further demonstrated by experiments with combinations of stresses. Moore et al. (1984) found that respiratory rate of mussels was not significantly affected by a three-day exposure to 20 $\mu\text{g/L}$ copper (111% of control rate) or 100 $\mu\text{g/L}$ phenanthrene (109% of control), but that a mixture of the two, sharply raised oxygen consumption (152% control) suggesting a synergistic effect. Calculation of maintenance energetic costs demonstrated an increase of 3% and 1.2% respectively, for mussels exposed to copper and phenanthrene when compared to maintenance cost of control mussels, respectively (Moore et al. 1984). The mixture caused an increase of 5.5% which again corresponds to a reduction in energy available for growth and reproduction over the long term.

Heart Rate

Another physiological function addressed is the effect of PAH exposure on heart rate. In mussels (*M. californianus*), this parameter was not affected by exposure to a nominal concentration of 1 mg/L of benzo[a]pyrene but was greatly reduced at 10 mg/L. After transfer to clean seawater, heart rate increased significantly before complete

recovery was achieved over three days (Sabourin and Tullis 1981). Exposure concentrations were not monitored in this study, however, the nominal concentrations are clearly unrealistic of environmental concentrations, since water solubility of benzo[a]pyrene is on the order of 2 µg/L.

2.2.2.2.3 Mixed Function Oxidase Activity

MFO activity in aquatic invertebrates has been reported in 18 marine species, including four phyla (Annelida, Arthropoda, Echinodermata, and Mollusca). It is composed of phospholipid, P-450 reductase and cytochrome P-450 and is located in the microsomal fractions associated with the stomach, hepatopancreas and green gland of crustaceans (Lee 1981).

With respect to the marine mussel, Mytilus edulis, there is disagreement among researchers as to whether or not this bivalve possesses a cytochrome P-450 dependent benzo[a]pyrene mono-oxygenase (Kurelec et al. 1985, 1986, Stegeman 1985). The evidence from the above studies suggests, however, that even if the MFO P-450 cytochrome is present, the metabolism of benzo[a]pyrene to the conventional 7,8- and 9,10-diol epoxides does not occur, and this is supported by a low measurable frequency of B[a]P adducts in a closely related species Mytilus galloprovincialis (Kurelec et al. 1987).

In contrast to marine organisms, there is only limited information regarding MFO activity in freshwater invertebrates.

Several lines of indirect evidence suggest MFO activity in freshwater invertebrates is also limited. Lu et al. (1977) used aquatic microcosms to expose the mosquito fish (Gambusia affinis), mosquito larvae (Culex pipiens quinquefasciatus) pond snail, (Physa sp.), and the cladoceran (D. magna) to benzo[a]pyrene. The BCF after 3 days was 930, 11536, 82231 and 134248 for each organism, respectively. The

notable difference in BCF between the fish and three invertebrates suggests the invertebrates have a poor ability to transform and depurate the PAH. In a related study, the mosquito fish, mosquito larvae and snail were assessed for ^{14}C -B[a]P accumulation over 3 days in the presence and absence of the MFO inhibitor piperonyl butoxide. For the mosquito fish in the absence of inhibitor, essentially all B[a]P existed in metabolite form. However, in the presence of MFO inhibitor, the parent B[a]P accounted for approximately 21%. For the mosquito larvae and snail, the parent compound accounted for 22 and 88%, respectively, of total ^{14}C radioactivity in the absence of inhibitor. When the MFO inhibitor was used, the value increased to 86% for mosquito larvae, again indicating a definite MFO inhibitor effect. However, the profile for snail remained essentially unchanged, indicating no inhibitory effect, and therefore little to no MFO activity (Lu et al. 1977).

Among the few studies directly addressing MFO activity in freshwater invertebrates, Khan et al. (1972) reported in vitro basal activity of a P-450-like MFO in the green gland of the crayfish Cambarus. The isolated microsomal fraction demonstrated epoxidation (aldrin, isodrin), hydroxylation (benzo[a]pyrene) and O-demethylation (methoxychlor). Although in vivo exposures were not addressed in the study, Khan et al. (1974) demonstrated in vitro MFO metabolism in freshwater crayfish. This suggests a potential for these organisms as a freshwater, invertebrate, environmental monitor for MFO inducing compounds. This would be especially useful in view of their .MT 5 restricted movement relative to fish, however, research on the in vivo inducibility of the MFO system in this organism would first be essential.

The available evidence suggests limited development of the MFO system in freshwater invertebrates and this is consistent with the high bioaccumulation of various PAH in invertebrates. With the possible exception of Cambrus, noted above, it would appear that use of MFOs for field monitoring could not be appropriately applied using these organisms.

2.2.2.3 Chronic Effects

2.2.2.3.1 Carcinogenesis

Research on the carcinogenic effects of PAH to aquatic invertebrates is limited to marine organisms. Field collections of the marine soft-shell clams (Mya arenaria) have associated industrial pollution with an increased prevalence of neoplastic lesions (Farley 1977). In some cases, the neoplasms have been associated with petroleum-derived hydrocarbon pollution, especially No. 2 Fuel Oil and JP-4 and JP-5 oils (Brown et al. 1979, Yevitch and Barszcz 1977). Both studies documented an incidence of tumors greater than 25% in soft-shell clams in contaminated areas. The cases were characterized by anaplastic, mitotically-active cells in connective tissue or gonadal follicles, with evidence of invasiveness and metastasis (Brown et al. 1979). In their 1977 study, Yevitch and Barszcz identified a group of hematopoietic neoplasms of gonadal origin which could have a different (viral) etiology.

Field collections of bryozoans in contaminated environments have been inconclusive. Powell et al. (1970) induced the development of abnormal bryozoan (Schizoporella unicornis) colonies after a 7 to 9 day exposure to coal-tar derivatives. However, Straughan and Lawrence (1975) collected a number of bryozoan species from Kelp exposed to oil seepage and found no evidence of ovicell hyperplasia.

2.2.2.3.2 Growth and Reproductive Effects

Growth

The increased standard metabolic rate in invertebrates exposed to PAH, previously discussed, suggests a decreased scope for growth may result. Nereis virens and N. succinea fed food contaminated by crude oil or benz[a]anthracene also exhibited a significant reduction in growth rates (Lee et al. 1981).

When eggs of Diaptomus clavipes were laid and incubated in the presence of acridine (0.32 to 3.2 mg/L), there was no observed effect on the development time of the eggs. Development times of the juvenile life stages (first naupliar stage to the adult stage) were significantly increased. Increasing the temperature from 16°C to 21°C evoked a threefold sensitization to acridine. Exposure to acridine had no significant effects on either first copepodid size or adult size (Cooney et al. 1984).

Reproduction

The effects of acridine, a component of synthetic fuel, on the copepod, Diaptomus clavipes, is among the more comprehensive evaluations of PAH effects on the reproduction of freshwater invertebrates found in the literature (Cooney et al. 1984). Among the parameters tested, rates of eggs and clutch production were the most sensitive. Concentrations higher than 1.0 mg/L completely inhibited the production of viable eggs at 16°C, while egg production ceased at 1.8 mg/L at 26°C. Clutch size and time to hatching were only affected at concentrations higher than 1.0 mg/L of acridine. The percentage of eggs hatching was more than 90% at all concentrations tested (0.3 to 1.0 mg/L acridine). The effect of acridine on Diaptomus reproduction was shown to be enhanced at elevated temperature with maximum acridine stress observed at 26°C (Cooney et al. 1984).

Parkhurst et al. (1981) investigated the chronic toxicity of acridine to the cladoceran, Daphnia magna, over a 28 day exposure. Several different end points to assess chronic toxicity were addressed, including long term survival, time to sexual maturity, and various measures of fecundity (Table 2.2.5). Of the six end points studied, the three associated with fecundity were the most sensitive indicators of chronic, sublethal impacts. The lowest chronic values as defined by Stephan et al. (1985) was 0.57 mg/L. Blaylock et al. (1985) also reported decreased fecundity in D. magna following a 14-day exposure to 1.25 mg/L acridine, which agrees well with the study of Parkhurst et al. (1981, Table 2.2.5).

TABLE 2.2.5
CHRONIC TOXICITY OF ACRIDINE TO Daphnia magna

Toxicity Criteria	NOEC (mg/L)	LOEC (mg/L)	Chronic Value (mg/L)
Survival	0.8	1.6	1.13
Development to primiparous instar	1.6	3.2	2.26
Sexual Maturity	0.8	1.6	1.13
No. of Broods	0.4	0.8	0.57
No. of Young/Brood	0.4 0.63	0.8 1.25	0.57 0.79 ^a
Young Produced/Female	0.4	0.8	0.57

Footnote: a - Data from Blaylock et al. 1985, all other data from Parkhurst et al. 1981)

Exposure of the calanoid copepod Eurytemora affinis for the duration of the adult life stage to naphthalene and alkylated derivatives (2-methylnaphthalene, 2,3,5-trimethylnaphthalene) for 29 days, resulted in significant reductions of the total number of nauplii produced, and a decrease of the mean brood size. Exposure to 10 µg/L of naphthalene resulted in a reduction of egg production to a rate of 50% of that observed in control animals. It was suggested that this effect on fecundity was related to the stage of egg development during exposure. Maximum effect would be associated with early stages of egg development (Ott et al. 1978).

The effect of the number of methyl groups on the toxicity of the above compounds cannot explain the observed differences between the response to the tested compounds. The most toxic compound was 2,3,5-trimethylnaphthalene, while naphthalene and 2-methylnaphthalene ranked second and third, respectively (Ott et al. 1978). It was not evident if a methanol carrier control was employed in the above study.

Exposure of Daphnia pulex at 20°C for up to 30 h to a water emulsion of Norman Well crude oil (1 µL/L water), a light crude having an aromatic content of 15-20%, decreased fecundity (Engelhardt et al. 1981a). However, 172 hours after transfer to clean water, the average brood size returned to control level. Although the dissolved concentration of carbon was not quantified, it was much lower than the nominal concentration since a large portion of the oil was floating at the water surface or present as oil particles (Englehardt et al. 1981, Wong et al. 1981).

Although limited, the information available for acridine, naphthalene and its derivatives clearly indicate the potential for reproductive impacts to several invertebrates which comprise an important trophic level in the aquatic food chain. Measures of fecundity appear to be the best indicator of reproductive effects and chronic impacts in general, with respect to the selected PAH studied. The data is also superior to the sublethal, acute and subacute data, from a regulatory perspective.

2.2.2.4 Toxicokinetics

2.2.2.4.1 Uptake and Bioconcentration

As a result of their low water solubility, PAH entering the aquatic ecosystem partition between the aqueous phase, suspended matter, sediments and aquatic biota. It is clearly established that various PAH present in the dissolved phase are efficiently accumulated by invertebrates. The uptake of PAH from the water by D. pulex is proportional to the lipophilicity of the individual compound (Table 2.2.6). This is consistent with the relationship that exists between membrane permeability and polarity of chemicals (Southworth et al. 1978a,b).

PAH are also accumulated in mussels (M. edulis). Dissected gills of mussels rapidly accumulated naphthalene and benzo[a]pyrene (Lee et al. 1972a). Whole organisms also accumulated PAH from the water (Pruell et al. 1986, Southworth et al. 1978a,b, Widdow et al. 1982).

Ingestion of contaminated food is another potentially important source of contamination. Research is required to further delineate this area since the information available to date is scarce and is mostly limited to marine invertebrates. Hard clam larvae (Mercenaria mercenaria) fed on benzo[a]pyrene contaminated diatoms (Thalassiosira pseudonana), demonstrated the efficiency of the trophic transfer between phytoplankton and a primary consumer. Within 24 hours of feeding on food containing 42.2 $\mu\text{g/g}$ benzo[a]pyrene, hard clam larvae had accumulated 18.6 $\mu\text{g/g}$ of benzo[a]pyrene and had not reached saturation. Only 56% of the accumulated dose through a given day of food transfer had been eliminated in 24 hours, and 71% in 48 hours, indicating the potential for bioconcentration (Dobrowsky and Epifanio 1980).

TABLE 2.2.6
BIOACCUMULATION OF SOME PAH BY D. pulex

Chemical	Molecular Weight	Log K _{OW}	BCF	Uptake Rate (µg/g h-1)	Elimination Rate (µg/gh-1)
Naphthalene	128	3.30	131	197	1.667
Phenanthrene	178	4.45	325	203	0.543
Anthracene	178	4.45	917	702	0.589
9-methylanthracene	192	5.12	4583	561	0.144
Pyrene	202	4.89	2701	1126	0.343
Benz[a]anthracene	228	5.60	10109	669	0.144
Perylene	252	6.06	7191	752	0.139

(From Southworth et al. 1978a)

Uptake of PAH from food was also reported in D. pulex; naphthalene was accumulated 6 times more rapidly via ingested food than via exposure to naphthalene in the water. However, phenanthrene, benz[a]anthracene and benzo[a]pyrene were accumulated more efficiently from the water (Trucco et al. 1983). The conditions necessary for accumulation through the food chain are clearly established, and therefore, the food web is a potential source of PAH for secondary consumers (Dabrowski and Epifanio 1980, Trucco et al. 1983).

Since many invertebrates are filter feeders and are sessile organisms, many live in close association with sediments. It would be worthy to investigate the importance of sediments as a source of PAH concentration.

In a survey of PAH pollution in the Great Lakes, some amphipod and oligochaete species were sampled and analyzed for PAH content. It was concluded that the benthic organisms obtained PAH from the water when PAH concentrations in the sediment were low. However, when sediment concentrations were high, the majority of PAH originated from the sediments (Eadie et al. 1983). Benthic organisms are an important source of PAH for bottom feeders and therefore represent an important link in mobilizing sediment associated PAH to other trophic levels.

Uptake of PAH from sediments was also shown to occur in marine organisms such as clams and shrimps (Varanasi et al. 1985). Mussels (M. edulis) collected in the industrialized bay of Yaquina Bay, Oregon, U.S.A. had a PAH content of 986 μg PAH/Kg, while specimens gathered from a more remote area averaged 273.9 μg PAH/Kg (Mix and Schaffer 1983).

Bioconcentration of PAH is also supported by their depuration kinetics. The biological half-life of most PAH is between 12 and 18 days in mussels (M. edulis) with the exception of fluoranthene (Table 2.2.7, Pruell et al. 1986). The measured elimination rate of most PAH in D. pulex was found to be inversely related to their lipophilicity (Southworth et al. 1978a,b).

TABLE 2.2.7
BIOLOGICAL HALF-LIFE OF SOME PAH IN MUSSELS (*M. edulis*)

Chemical	Depuration Half-Life (days)
Fluoranthene	29.8
Benz[a]anthracene	17.8
Chrysene	14.2
Benzo[b]fluoranthene	16.9
Benzo[k]fluoranthene	11.9
Benzo[e]pyrene	14.4
Benzo[a]pyrene	15.4

(From Pruell et al. 1986)

The uptake of a chemical is affected by various modifying factors such as temperature, season, size and this is discussed in greater depth in Section 2.4. The presence of food enhanced the uptake of PAH from water by mussels. Mussels exposed to crude oil WSF in the presence of algal food had a higher body PAH concentration than when exposed to the WSF only. It was suggested that the presentation of algal food, and the reduction in clearance rate induced by WSF (Widdows et al. 1982) accounted for the observed higher tissue concentrations.

From the previous discussion, it is clear that aquatic invertebrates are efficient in extracting PAH from their environment and that elimination occurs relatively slowly. Consequently, conditions for bioconcentration of PAH are established. *D. pullex* bioconcentrate PAH from the water with an efficiency that is proportional to the log octanol-water partitioning coefficient of the chemical (Southworth et al. 1978a,b, Trucco et al. 1983.) which was shown to be a good

indicator of the bioconcentration potential. The relationship is described by the following equation:

$$\text{Log BCF (24-h)} = 0.7520 \log K_{ow} - 0.4362 \quad (2.2-1)$$

where: Log BCF = logarithm of the bioconcentration factor

Log K_{ow} = logarithm of the octanol-water partition coefficient
(Southworth et al. 1978b).

PAH bioconcentration by D. pulex is then explainable in terms of lipid/water partitioning. The measured bioconcentration factors ranges from 131 (naphthalene) just over 10,000 (benz[a]anthracene) (see Table 2.2.6).

The bioconcentration of PAH from water has also been reported in other invertebrates. Mussels (M. edulis) exposed for 40 days to resuspended sediments of the Providence River (Rhode Island, U.S.A.), bioconcentrated PAH. Bioconcentration factors calculated against the freely dissolved concentrations showed a better correlation than BCFs calculated against the particulate phase. This suggests that mussels accumulated most of the PAH from the dissolved phase. Equilibrium between the tissue concentrations and the dissolved phase was achieved within 20 days of exposure for the four to six-ring compounds. For the series of PAH tested, the ranking by decreasing importance of bioconcentration factor, is as follows: chrysene > benzo[b]fluoranthene > fluoranthene > benzo[e]pyrene and benz[a]anthracene. Again higher bioconcentration capacity was observed with the increasing lipophilicity of the compounds and is described by the following equation (Pruell et al. 1986):

$$\text{Log BCF} = 1.73 + 0.594 \text{ Log}K_{ow} \quad (2.2-2)$$

It is evident that invertebrates readily bioconcentrate PAH from their environment. Because mussels are sessile animals and can't eliminate accumulated PAH when transferred to PAH-free water, their utilization as a biomonitoring model is justified.

2.2.2.4.2 Tissue Distribution

PAH accumulation rate was shown to differ between tissues. Concentrations of total hydrocarbons observed in the digestive glands of North Sea crude oil WSF - exposed mussels were about 2.5 times higher than in other tissues (Widdows et al. 1982). The bioconcentration factor (BCF) reported for phenanthrene-exposed mussels was 200-300 in the digestive gland, 210-230 for the mantle, and 60-70 for the remaining tissues (Moore et al. 1984). The significance of the digestive gland in bioconcentration could partially be explained by its important role in the uptake of PAH, although some authors (Widdows et al. 1982) believe the differences in lipid content between the different organs to be a more likely explanation.

Adult spot shrimp (Pandalus platyceros) accumulated naphthalene, methyl-naphthalene and C2 and C3 substituted naphthalene in both the thorax and the abdomen (Sanborn and Malins 1980). The results of this study indicates a potential hazard to the human consumers of the shrimp.

The presence of an important macronuclear pool for benz[a]anthracene is suspected in N. virens. In a study using radioactive C14, only 2-23% of the [C14-12] benz[a]anthracene was found as the parent molecule, whereas the majority of the compound was in the form of metabolic products. The presence of a large proportion of the radioactivity that was neither solvent or water soluble suggests an important macromolecular pool (McElroy 1985). Similarly, the presence of a "slow" phase in the depuration kinetics of anthracene in D. pulex

as demonstrated by a three compartment elimination model, suggests a more tightly bound compartment containing about 8% of the residue (Herbes and Risi 1978). The components of this reservoir still have to be identified. Their identification might provide a better understanding of the modes of action of anthracene and benz[a]anthracene since the compounds can elicit toxicity by binding to DNA, RNA, enzymes or structural proteins.

2.3 AQUATIC PLANTS

2.3.1 Lethal Toxicity

There is little information available on the acute lethal toxicity of PAH to algae, possibly because the concentrations required for lethality are higher than those likely to occur in the biosphere (Table 2.3.1). However, a study by Høldal et al. (1984) detected a lethal response of Dunaliella bioculata to 9,10-epoxy-9,10-dihydroxyphenanthrene. The EC50 (really a LC50) for this compound was 0.55 mg/L, with a 95% CI of 0.3 - 2.5. The authors suggest that this EC50 value is probably conservative, as 9,10-epoxy-9,10-dihydroxyphenanthrene is unstable, and was likely degraded during the period required to measure lethality. The purpose of this study was to compare the relative toxicities of a group of structurally similar compounds. The epoxy hydrocarbons (of which this compound is one) are highly reactive. They are electrophilic, tending to react with nucleophilic groups, such as those found in DNA, RNA or proteins, which is the basis for their toxic, mutagenic and carcinogenic effects.

Soto et al. (1975a) determined that exposure of Chlamydomonas angulosa to naphthalene in a closed system (30.12 mg/L) resulted in 97 to 98% cell death. It is not known whether the surviving cells represent tolerant individuals, or whether an LC100 had not been reached. An open system, allowing volatilization of the naphthalene, resulted in a lower lethality (61%). Kauss and Hutchinson (1975) determined that the LC50 for Chlorella vulgaris exposed to naphthalene was 33 mg/L, although their highest experimental level was 30 mg/L.

The range of values represented by these latter lethality studies (30 to 33 mg/L) are at the limit of freshwater solubility for naphthalene (34.4 mg/L) (Bohon and Claussen 1951) so it is unlikely that these concentrations would be reached in the ambient aquatic environment.

Table 2.3.1: Lethal Toxicity of PAH to Algae

Chemical	Test Organism	Life Stage or Size	Method	Response Variable	Effect (mg/l)	Exposure Concentration	Exposure Duration	Reference
Naphthalene	<i>Chlamydomonas angulosa</i>	Active culture	CL	Lethality in closed system	97 to 98% cell death	30.11	3 to 7 days	Soto et al., 1975a
				Lethality in open system	61% cell death	30.11	1 day	Soto et al., 1975a
	<i>Chlorella vulgaris</i>	Active culture	CL	LC50 for cell number	33 mg/L	0 to 30	1 to 10 days	Fauss and Hutchinson, 1975
9,10-epoxy 9,10-dihydroxy Phenanthrene	<i>Dunaliella bioculata</i>	Active culture	CL	LC50	0.55 µg/L	-	50 to 72 hours	Heldal et al., 1984

However, the observed LC50 for 9,10-epoxy-9,10-dihydroxyphenanthrene was lower (0.55 mg/L), and was likely an over-estimation. This suggests that either this compound is 100 times as toxic as naphthalene, or that D. bioculata is 100 times more sensitive than A. flos-aquae. Either of these may be the case, and it demonstrates that PAH sensitivity is species (both algal and chemical) dependent.

2.3.2 Sublethal Toxicity

2.3.2.1 Subacute Effects on Algal Growth and Physiological Processes

The studies in this section describe the effects of PAH on the growth and physiological processes of algae (Table 2.3.2). In a few studies, EC50 for cell growth (i.e. decrease in cell numbers) have been calculated, but generally the percent change in growth for one or more doses of the PAH, relative to the control is reported. There are no mathematical dose response relationships presented. Metabolic processes receive less attention, and are limited to discussions of nitrogen fixation and photosynthesis. Generally speaking, the concentrations of PAH tested (usually only one or two) reduced growth of filamentous algae (i.e. Anabaena sp.) to some extent. In contrast, a study by Bastian and Toetz (1982) which examined the effect of many PAH on Anabaena flos-aquae, demonstrated that naphthalene in concentrations of 3.17 to 30.4 mg/L stimulated growth, with a maximum stimulation of 50% at 15.2 mg/L. Growth was measured after 14 days exposure in open flasks, and the naphthalene was lost completely from the medium within seven days. This suggests that the growth stimulation was in response to a very short exposure to naphthalene, as opposed to a 14 day exposure. All of the studies in this section were primary, or very good secondary, although most of them did not address the constancy of PAH concentration during exposure. These PAH included anthracene, benz[a]anthracene, benzo[a]pyrene, chrysene, fluoranthene, fluorene, naphthalene and phenanthrene.

Table 2.1.2: Subacute Effects on Algal Growth and Physiological Processes (Continued)

Chemical	Test Organism	Life Stage or Size	Method	Response Variable	Effect	Exposure Concentration (mg/L)	Exposure Duration	Reference
Acenaphthene	<i>Anabaena flos-aquae</i>	Active culture	CU	Nitrogen fixation	no effect	4.1 to 4.6	2 hours	Hastan and Tøet, 1985
	<i>Antithamion olivula</i>	Sporelings	CU	Cell growth	increase of 5%	0 to 1.6	14 days	Hastan and Tøet, 1985
		Sporelings	CU	Cell growth	decrease of 5%	0.2	30 to 60 minutes	Bonet, 1974
	<i>A. olivula</i>	Sporelings	CU	Cell growth	increase of 15%	0.1	24 to 60 minutes	Bonet, 1974
Acriflame	<i>Selenastrum capricornutum</i>	Active culture	CU	EC50 for decreased cell growth	6.9 mg/L	0 to 11	24 hours	Blaslow et al., 1955
	<i>A. olivula</i>	Sporelings	CU	Cell growth	decrease of 5%	9.3	30 to 60 minutes	Bonet, 1974
Atrazine	<i>Chlamydomonas angulosa</i>	Logarithmic growth	CU	EC50 for photosynthesis	.567 mg/L	0.042	3 hours	Hutchinson et al., 1980
	<i>Chlorella vulgaris</i>	Logarithmic growth	CU	EC50 for photosynthesis	.356 mg/L	0.042	3 hours	Hutchinson et al., 1980
	<i>S. capricornutum</i>	Exponential growth	CU	Log ₁₀ BCF	3.89	-	-	Milbrink, 1987
Atrazine	<i>S. capricornutum</i>	Exponential growth	CU	Uptake rate	9.112 min	-	-	Milbrink, 1987
	<i>S. capricornutum</i>	Active culture	CU	EC50 for decreased cell growth	17.8 mg/L	.0012 to 17.6	4 to 7 days	Good et al., 1984
Atrazine	<i>A. olivula</i>	Sporelings	CU	Cell growth	no effect	0.3	30 to 60 minutes	Bonet, 1974

Table 2.3.2: Subacute Effects on Algal Growth and Physiological Processes (cont'd)

Chemical	Test Organism	Life Stage or Size	Method	Response Variable	Effect	Exposure Concentration (µg/L)	Exposure Duration	Reference
Benzalanthracene	<i>S. caoricorotum</i>	Active culture	CL	EC50 for decreased cell growth	72.2 µg/L	.0023 to 22.8	4 to 7 days	Cody et al., 1984
3,10 dimethyl Benzalanthracene	<i>A. flos-aquae</i>	Active culture	CL	Cell growth	decrease of 46%	0 to .013	14 days	Bastian and Toett, 1982
	<i>A. flos-aquae</i>	Active culture	CL	Nitrogen fixation	decrease of 30%	0.030	2 hours	Bastian and Toett, 1985
	<i>A. pluvula</i>	Sporelings	CL	Cell growth	1.6 to 2.3 fold increase	.01 and 0.1	96 hours	Bones and Corner, 1982
	<i>A. pluvula</i>	Sporelings	CL	Cell growth	2.3 fold increase	.01 and 0.1	24 hours	Bones and Corner, 1982
Benzidine	<i>A. pluvula</i>	Sporelings	CL	Cell growth	increase of 12%	0.3	36 to 60 minutes	Shen, 1974
	<i>S. caoricorotum</i>	Active culture	CL	EC50 for decreased cell growth	0.025 µg/L	0.0025 to .25	4 to 7 days	Cody et al., 1984
<i>A. pluvula</i>	<i>A. pluvula</i>	Sporelings	CL	Cell growth	1.5 fold increase in growth	.01 to .1	96 hours	Bones and Corner, 1982
	<i>A. pluvula</i>	Sporelings	CL	Cell growth	increase of 34%	3	30 minutes	Bones 1974
Chrysene	<i>Porphyra tenera</i>	Sporelings	CL	Cell proliferation	increase of 40%	1	80 to 320 minutes	Bones 1974
	<i>A. flos-aquae</i>	Active culture	CL	Nitrogen fixation	decrease of 17%	0.014	2 hours	Bastian and Toett, 1985
<i>A. pluvula</i>	<i>A. pluvula</i>	Sporelings	CL	Cell growth	decrease of 35%	0.0019	14 days	Bastian and Toett, 1982
	<i>A. pluvula</i>	Sporelings	CL	Cell growth	increase of 5%	0.3	30 to 60 minutes	Bones 1974
Fluoranthene	<i>A. flos-aquae</i>	Active culture	CL	Nitrogen fixation	decrease of 28%	.435	2 hours	Bastian and Toett, 1985

Table 2.3.2: Subacute Effects on Algal Growth and Physiological Processes (cont'd)

Chemical	Test Organism	Life Stage or Size	Method	Response Variable	Effect	Exposure Concentration (mg/L)	Exposure Duration	Reference
Fluorene	<i>A. flos-aquae</i>	Active culture	CL	Cell growth	decrease of 100%	0 to 0.25	14 days	Bastian and Toetz, 1982
				Nitrogen fixation	decrease of 50%	0.512	2 hours	Bastian and Toetz, 1985
	<i>Chlorella</i> sp.	Cultured specimens	CL	Cell growth	decrease of 65%	0 to 1.9	14 days	Bastian and Toetz, 1982
	<i>Chlorella</i> sp.	Cultured specimens	CL	EC50 for 'production' (pre-emergent exposure)	20.3 mg/L	0.2 to 25.0	21 days	Finner et al., 1985
				EC50 for 'production' (post-emergent exp)	135.0 mg/L	3.5 to 35.0	21 days	Finner et al., 1985
	<i>Quasipeltia bicuspidata</i>	Active culture	CL	EC50 for decreased growth	0.55	0	5 to 72 hours	He, 1981, 1982
2 Methylanthracene	<i>S. caricorodum</i>	Active culture	CL	EC50 for 'production'	3.4 mg/L	3.0 to 5.0	36 hours	Finner et al., 1985
				Threshold for chlorophyll/cell number reduction	1.67 (13.33 mg/L)	0 to 5	7 days	Finner et al., 1985
	<i>A. plumula</i>	Sporulines	CL	Cell growth	decrease of 12%	0.3	30 to 60 minutes	Boney, 1974
3 Methylcholanthrene	<i>A. plumula</i>	Sporulines	CL	Cell growth	increase of 88% and 55%	0.01 and 0.1	4 days	Boney and Corner, 1982
				Cell growth	increase of 14% and 28%	0.01 and 0.1	4 days	Boney and Corner, 1982
	<i>Callithamnion tetricum</i>	Sporulines	CL	Cell growth	increase of 23% and 28%	0.1	4 days	Boney and Corner, 1982
Menthylene	<i>A. flos-aquae</i>	Active culture	CL	Nitrogen fixation	decrease of 16%	2.08	2 hours	Bastian and Toetz, 1982
				Cell growth	increase of 58%	0 to 30.4	14 days	Bastian and Toetz, 1982
	<i>Saccharina non-repens</i>	Sporulines	CL	Cell growth	increase of 23% and 28%	0.1	4 days	Boney and Corner, 1982

Table 2.3.2: Subacute Effects on Algal Growth and Physiological Processes (cont'd)

Chemical	Test Organism	Life Stage or Size	Method	Response Variable	Effect	Exposure Concentration (mg/L)	Exposure Duration	Reference
1 acetyl Naphthalene	<i>Chlamydomonas angulosa</i>	Active culture	CL	Photosynthesis	decrease of 30%	3.17 to 25.4	2 to 16 days	Soto et al., 1975b
	<i>C. angulosa</i>	Logarithmic growth	CL	EC50 for photosynthesis	8.96 mg/L	0 to 31.7	3 hours	Hutchinson et al., 1980
	<i>C. vulgaris</i>	Logarithmic growth	CL	EC50 for photosynthesis	16.6 mg/L	0 to 31.7	3 hours	Hutchinson et al., 1980
	<i>Dunaliella</i> sp.	Active culture	CL	EC50 for ^{14}C fixation	3 mg/L	0 to 7	20 min	Vanderaueken and Abern., 1976
	<i>Fragilaria</i> sp.	Active culture	CL	EC50 for ^{14}C fixation	5.5 mg/L	1 to 7	20 min	Vanderaueken and Abern., 1976
	<i>Monocargis lutheri</i>	Active culture	CL	EC50 for ^{14}C fixation	2.4 mg/L	1 to 7	20 min	Vanderaueken and Abern., 1976
	<i>S. caudicatum</i>	Logarithmic growth	CL	Log ₁₀ RCF	4.10	10.0	24 hours	Cassidy et al., 1983
	<i>C. angulosa</i>	Logarithmic growth	CL	EC50 for photosynthesis	1.70 mg/L	0 to 28.5	3 hours	Hutchinson et al., 1980
	<i>C. vulgaris</i>	Logarithmic growth	CL	EC50 for photosynthesis	4.69 mg/L	0 to 28.5	3 hours	Hutchinson et al., 1980
	<i>C. angulosa</i>	Logarithmic growth	CL	EC50 for photosynthesis	3.55 mg/L	0 to 25.4	3 hours	Hutchinson et al., 1980
2 acetyl Naphthalene	<i>C. vulgaris</i>	Logarithmic growth	CL	EC50 for photosynthesis	7.21 mg/L	0 to 25.4	3 hours	Hutchinson et al., 1980
	<i>Perrine</i>	Sporelings	CL	Cell growth	no effect	0.3	30 to 60 minutes	Boney, 1974

Table 2.3.2: Subacute Effects on Algal Growth and Physiological Processes (cont'd)

Chemical	Test Organism	Life Stage or Size	Method	Response Variable	Effect	Exposure Concentration $\mu\text{g/L}$	Exposure Duration	Reference
Phenanthrene	<i>A. flos-aque</i>	Active culture	CU	Nitrogen fixation	decrease of 40%	0.134	2 hours	Bastian and Toetz, 1985
	<i>A. flos-aque</i>	Active culture	CU	Cell growth	decrease of 15%	0 to 1.26	14 days	Bastian and Toetz, 1982
	<i>C. angulosa</i>	Logarithmic growth	CU	EC50 for photosynthesis	.890 $\mu\text{g/L}$	0 to 1.29	3 hours	Hutchinson et al., 1980
	<i>C. vulgaris</i>	Logarithmic growth	CU	EC50 for photosynthesis	1.16 $\mu\text{g/L}$	0 to 29.5	3 hours	Hutchinson et al., 1980
Phenylacetalene	<i>S. capricornutum</i>	Logarithmic growth	CU	Log ₁₀ BCF	4.38	1.0	24 hours	Cassidy et al., 1983
	<i>A. pluvula</i>	Sporlings	CU	Cell growth	no effect	0.3	30 to 60 minutes	Boney, 1974
	<i>A. flos-aque</i>	Active culture	CU	Nitrogen fixation	increase of 1%	0.085	2 hours	Bastian and Toetz, 1985
	<i>A. flos-aque</i>	Active culture	CU	Cell growth	no effect	0 to 0.130	14 days	Bastian and Toetz, 1982
Pyrene	<i>A. pluvula</i>	Sporlings	CU	Cell growth	no effect	0.3	30 to 60 minutes	Boney, 1974
	<i>C. angulosa</i>	Logarithmic growth	CU	EC50 for photosynthesis	.202 $\mu\text{g/L}$	0 to 0.135	3 hours	Hutchinson et al., 1980
	<i>C. vulgaris</i>	Logarithmic growth	CU	EC50 for photosynthesis	.303 $\mu\text{g/L}$	0 to 0.135	3 hours	Hutchinson et al., 1980
	<i>S. capricornutum</i>	Logarithmic growth	CU	Log ₁₀ BCF	4.56	0.5	24 hours	Cassidy et al., 1983
Triphenylene	<i>A. pluvula</i>	Sporlings	CU	Cell growth	decrease of 1%	0.3	30 to 60 minutes	Boney, 1974

Table 2.3.2: Subacute Effects on Algal Growth and Physiological Processes (cont'd)

Chemical	Test Organism	Life Stage or Size	Method	Response Variable	Effect	Exposure Concentration (mg/L)	Exposure Duration	Reference
Mixture of: Naphthalene, Phenanthrene and Pyrene	<i>S. caudicatus</i>	Lodgeritic growth	CL	LD ₅₀ BCP	4.25 4.57 4.75	2.0 1.0 0.5	24 hours	Cassidy et al., 1983

Footnotes:

* CL = cells in liquid culture

The effect of many of these compounds on laminar algae (i.e. Antithamnion sp.) was to increase cell division (Boney 1974, Ishio et al. 1972a,b). These compounds included acetylaminofluorene, benzanthracene, benzidine (9,10-methyl and 6-methyl), benzo[a]pyrene, chrysene, methyl anthracene, and methylcholanthrene. These differences in response between filamentous and laminar algal response may reflect a significant role for species as a modifying factor of PAH toxicity. It is interesting to note that the increased cell division by laminar algae occurred at higher concentrations (chrysene 0.296 mg/L; phenanthrene 0.303 mg/L) than growth reductions of filamentous algae (chrysene 0.0139 mg/L; phenanthrene 0.134 mg/L).

The difference in species sensitivity is probably due to the different growth forms of these algae. The laminar algae are multilayered, multicellular, and generally lack an internal transport system. It is likely that the internal cells of these algae receive a much lower dose of PAH than the external cells, due to the diffusion resistance posed by plant cell membranes and walls. There is some direct evidence for this; external cells have been reported to show a greater degree of growth aberrations than internal cells (Boney 1974, Boney and Corner 1962). Similar responses have been noted in apical cells, which in laminar algae are the oldest cells and so have been exposed the longest. Filamentous algae, on the other hand, expose a far greater percentage of their cellular surface to the toxicant, resulting in a potentially greater dose per cell. A second difference between these two types of algae is that the vegetative laminar algal cells are differentiated (e.g. photosynthetic, cortical) whereas the vegetative filamentous algal cells are not. Cells with different function may have different sensitivities. The specific processes of the cell (for example, the reducing power often associated with photosynthetic cells) may reduce the impact of a toxicant on that cell. Boney and Corner (1974) have suggested that the tendency of a PAH to induce cell division in laminar algae may be a good predictor of its potential carcinogenicity in animals, although they do not support this with any studies of correlation. Given the lack of

support for the extrapolation of animal data to human toxicity modeling, it is difficult to see a similar role for algae in carcinogenicity screening in the near future. It is also worth noting that the exposure concentrations for several of the PAH are in excess of their solubility in freshwater at 25°C (Mackay and Shiu, 1977). It is therefore unlikely that the toxic responses as described would be observed in most ambient aquatic environments in Ontario.

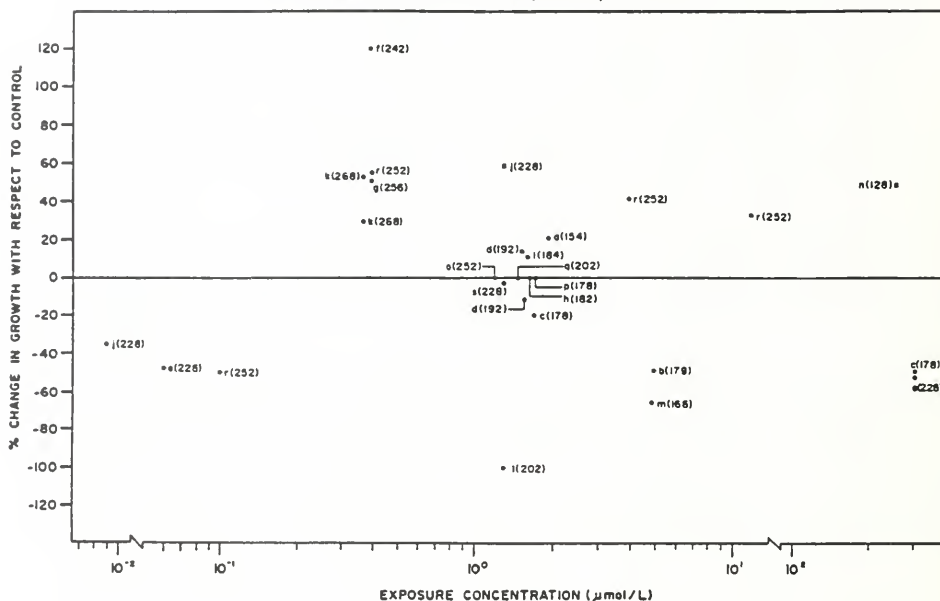
The growth effects data for algae have been summarized in Figure 2.3.1, which demonstrates the relationship between concentration and effect; all PAH are plotted together, and the MW of the PAH at each data point is labeled. This figure suggests that there is little relationship between concentration ($\log_{10} \mu\text{mol/L}$) and percent change in growth. However, when the exposure duration is taken into account (Figure 2.3.2) there appears to be a weak relationship between dose ($\log_{10} (\text{concentration}[\mu\text{mol/L}] \times \text{duration}[\text{days}])$) and effect. In general, higher doses result in greater growth reduction.

These data were examined for mathematical relationships; the germane parameters are included on the figures. In summary, linear relationships were statistically significant, but the coefficients of correlation were low. Included here are data for many species from various authors; most of the studies were conducted under unique conditions, making a comparison difficult.

The effect of PAH on nitrogen fixation (Bastian and Toetz, 1985) has been investigated using Anabaena flos-aquae: nitrogen fixation was suppressed by most of the chemicals tested. The duration of exposure for this study was short (2 h) and it is difficult to know whether this suppression of nitrogen fixation would persist if the exposure was chronic. However, if it did, such a suppression would likely result in a loss of vitality and productivity of the algal cells. The same applies to a study of photosynthesis in Chlamydomonas angulosa (Soto et al. 1975b). This study demonstrated that photosynthesis was depressed by 90 to 100% following a 2 h exposure to naphthalene at

Figure 2.3.1

CHANGE IN ALGAL GROWTH RATE AS A FUNCTION OF PAH EXPOSURE CONCENTRATION. LETTERS INDICATE DATA POINT AND COMPOUND. NUMBERS IN PARENTHESIS INDICATE MOLECULAR WEIGHT ($y = 2.32 - 12.6x$, $r^2 = 0.07$, $P = 0.17$)

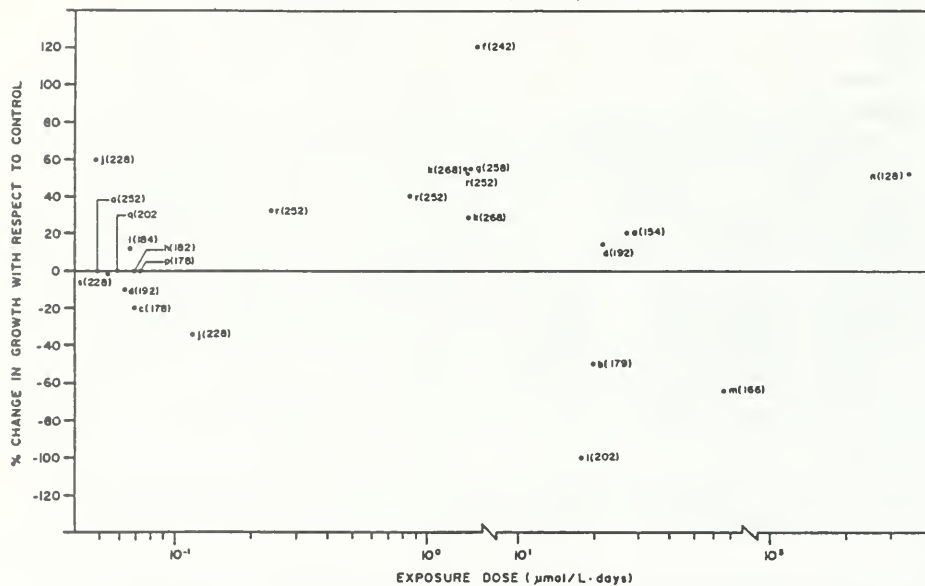


REFERENCES TO DATA POINTS FOR TABLES 2.3.1 AND 2.3.2

DATA POINT	CHEMICAL	REFERENCE
a(154)	Acenaphthene	Boney 1974
b(179)	Acridine	Bastian and Toeltz 1982
c(178)	Anthracene	Blaylock et al 1985
d(192)	Methylanthracene	Boney 1974, Cody et al 1984
e(228)	Benz[a]anthracene	Boney 1974
f(242)	Methylbenz[a]anthracene	Cody et al 1984
g(256)	Dimethylbenz[a]anthracene	Boney and Corner 1962
h(182)	Azobenzene	Boney and Corner 1962
i(184)	Benzidine	Boney 1974
j(228)	Chrysene	Boney 1974
k(268)	Methylcholanthrene	Bastian and Toeltz 1982
l(202)	Fluoranthene	Boney 1974
m(166)	Fluorene	Boney and Corner 1962
n(128)	Naphthalene	Bastian and Toeltz 1982
o(252)	Perylene	Bastian and Toeltz 1982
p(178)	Phenanthrene	Boney 1974
q(202)	Pyrene	Bastian and Toeltz 1985
r(252)	Benzo[a]pyrene	Boney 1974
s(226)	Triphenylene	Cody et al 1984
		Boney and Corner 1962
		Boney 1974
		Boney 1974

Figure 2.3.2

CHANGE IN ALGAL GROWTH RATE AS A FUNCTION OF PAH EXPOSURE DOSE ($\mu\text{mol/L} \cdot \text{days}$). LETTERS INDICATE DATA POINT AND COMPOUND NAME (ACCORDING TO FIGURE 2.3.1). NUMBERS IN PARENTHESIS INDICATE MOLECULAR WEIGHT ($\gamma = 8.42 - 13.0x$, $r^2 = 0.12$, $P = 0.10$).



concentrations as low as 3.17 mg/L. This was in a closed system, so no volatilization of PAH occurred. When cells were exposed to naphthalene saturated medium, the rate at which the cells recovered full photosynthetic capacity (once the flask was opened) was inversely related to the number of days of incubation in the closed system. Cells incubated with naphthalene for one and seven days regained photosynthetic rates equal to control rates within a few days. It appears that cells from two, three, four and eight day incubation periods were not measured long enough to detect 100% recovery, although the slopes of the rates indicated that it was likely. Vandermeulen and Ahern (1976) demonstrated that the EC50's for ^{14}C fixation in three algal species exposed to naphthalene ranged between 3 and 5 mg/L. They also demonstrated that photosynthesis in Monochrysis lutheri recovers from exposure to 5.4 mg/L naphthalene within 5 hours after removal of the naphthalene. There were no changes in chlorophyll a, nor the phaeophytin concentration in response to naphthalene, although the ATP concentration was reduced. The authors concluded suggested the following:

- a) trace amounts of naphthalene may affect algal species,
- b) inhibition of carbon fixation may have two modes, one for each of the high and low exposure concentrations,
- c) the inhibition of carbon fixation is reversible in exposures of less than 12 hours,
- d) the inhibition of carbon fixation may be a symptom of the primary effect of naphthalene on algae, such as reduction of ATP concentration.

Algae play an important role in the aquatic biosphere as primary producers through carbon fixation, and as oxygenators. It is therefore desirable that their physiological processes (including growth) not be significantly impaired. In consequence, environmental standards for PAH should be geared towards protecting these processes

as opposed to preventing lethality. The limitation of the current data base, is that much literature details the effects of short term, high concentration exposures, from which it is impossible to infer the effect of long term, lower concentration exposures.

2.3.2.2 Toxicokinetics

2.3.2.2.1 Bioconcentration of PAH in Algae

The literature which forms the basis for this section is survey data; that is, algae collected from natural ecosystems exposed to ambient water (Payer et al. 1975, Knutzen and Sortland 1982). Scandinavia, industrialized Europe and the far East are the areas which were sampled. Although as expected, higher concentrations were found in the sampling sites closest to industrialization, PAH were also detected in areas distant from known sources. The PAH profiles were somewhat species dependent, indicating selectivity of uptake or metabolism, or both. Fluoranthene, naphthalene, pyrene, phenanthrenes and anthracene were the dominant PAH, present in tissue at concentrations varying from traces to 900 $\mu\text{g/kg}$ on a plant dry weight basis (Table 2.3.3). None of the studies in this section are considered to be primary, as the exposure doses are not known.

The frequency distribution of concentrations of carcinogenic PAH was log-normal (the most frequent PAH concentration was near zero), whereas the frequency distribution of non-carcinogenic PAH was normally distributed (Payer et al. 1975). The authors suggest that a normal distribution of concentration frequency is evidence that non-carcinogenic PAH are synthesized de novo, as the frequency of concentration is weighted towards the median, indicating the accumulation of endogenous compounds. The log-normal distribution, on the other hand, suggests environmental deposition of carcinogenic PAH, the log-normal distribution being attributed to variability in duration of exposure to emissions. The lack of de novo synthesis of carcinogenic compounds is a reasonable strategy from an evolutionary standpoint.

Table 2.1.3: Observed Tissue Concentrations of PAHs in Algae as a Result of Ambient Environmental Exposure

Chemical	Test Organism	Life Stage or Site	Method	Tissue Concentration ($\mu\text{g/g}$)	Reference
Benz[a]anthracene	<i>Ascopyllus nodosus</i>	Ambient collection	CL ^a	nd ^b to 21	Eutizen and Sorlland, 1982
	<i>Ceramium rubrum</i>	Ambient collection	CL	4	Eutizen and Sorlland, 1982
	<i>Fucus serratus</i>	Ambient collection	CL	3 to 40	Eutizen and Sorlland, 1982
	<i>Fucus vesiculosus</i>	Ambient collection	CL	2 to 73	Eutizen and Sorlland, 1982
	<i>Laminaria saccharina</i>	Ambient collection	CL	5	Eutizen and Sorlland, 1982
Benzo[b]fluoranthene	<i>A. nodosus</i>	Ambient collection	CL	nd to 12	Eutizen and Sorlland, 1982
	<i>C. rubrum</i>	Ambient collection	CL	5	Eutizen and Sorlland, 1982
	<i>F. serratus</i>	Ambient collection	CL	4 to 31	Eutizen and Sorlland, 1982
	<i>F. vesiculosus</i>	Ambient collection	CL	nd to 76	Eutizen and Sorlland, 1982
	<i>L. saccharina</i>	Ambient collection	CL	4	Eutizen and Sorlland, 1982

Table 2.3.3: Observed Tissue Concentrations of PAHs in Algae as a Result of Ambient Environmental Exposure

Chemical	Test Organism	Life Stage or Size	Method	Tissue Concentration (ng/gp.d)	Reference
Benzol[a]fluoranthene	<i>S. acutus</i>	Ambient collection	CL	2.4 to 36	Payer et al. 1975
	<i>A. nodosus</i>	Ambient collection	CL	nd to 12	Knutzen and Sorlland, 1982
	<i>C. rubrum</i>	Ambient collection	CL	6	Knutzen and Sorlland, 1982
Benzol[a]fluoranthene	<i>F. serratus</i>	Ambient collection	CL	4 to 15	Knutzen and Sorlland, 1982
	<i>F. vesiculosus</i>	Ambient collection	CL	nd to 14	Knutzen and Sorlland, 1982
	<i>L. saccharina</i>	Ambient collection	CL	4	Knutzen and Sorlland, 1982
Benz[e]acephenanthrylene	<i>Scenedesmus acutus</i>	Ambient collection	CL	6 to 85	Payer et al. 1975
	<i>A. nodosus</i>	Ambient collection	CL	trace to 12	Knutzen and Sorlland, 1982
Benzol[a]pyrene	<i>C. rubrum</i>	Ambient collection	CL	5	Knutzen and Sorlland, 1982
	<i>F. serratus</i>	Ambient collection	CL	2 to 20	Knutzen and Sorlland, 1982
	<i>F. vesiculosus</i>	Ambient collection	CL	nd to 64	Knutzen and Sorlland, 1982

Table 2.3.3: Observed Tissue Concentrations of PBAs in Algae as a Result of Ambient Environmental Exposure (cont'd)

Chemical	Test Organism	Life Stage or Size	Method	Tissue Concentration ($\mu\text{g/g}$)	Reference
Benzol[a]pyrene (Cont'd)	<i>L. saccharina</i>	Ambient collection	CL	4	Eutizen and Sorland, 1982
	<i>S. acutus</i>	Ambient collection	CL	1.39 to 39.5	Peter et al., 1975
Benzole[pyrene	<i>A. nodosum</i>	Ambient collection	CL	trace to 8	Eutizen and Sorland, 1982
	<i>C. rubrum</i>	Ambient collection	CL	14	Eutizen and Sorland, 1982
	<i>F. serratus</i>	Ambient collection	CL	7 to 30	Eutizen and Sorland, 1982
	<i>F. vesiculosus</i>	Ambient collection	CL	ad to 100	Eutizen and Sorland, 1982
Benzol[ghi]perylene	<i>L. saccharina</i>	Ambient collection	CL	7	Eutizen and Sorland, 1982
	<i>A. nodosum</i>	Ambient collection	CL	ad	Eutizen and Sorland, 1982
	<i>C. rubrum</i>	Ambient collection	CL	4	Eutizen and Sorland, 1982
	<i>F. serratus</i>	Ambient collection	CL	ad to 79	Eutizen and Sorland, 1982
	<i>F. vesiculosus</i>	Ambient collection	CL	12 to 554	Eutizen and Sorland, 1982

Table 2.1.1: Observed Tissue Concentrations of PAHs in Algae as a Result of Ambient Environmental Exposure (cont'd)

Chemical	Test Organism	Life Stage or Size	Method	Tissue Concentration ($\mu\text{g/gwt}$)	Reference
Benzo(b)perylene (Cont'd)	<i>L. saccharina</i>	Ambient collection	CL	nd	Knutzen and Sorland, 1982
	<i>S. moultus</i>	Ambient collection	CL	3.89 to 52.1	Payer et al., 1975
Chrysene/triphenylene	<i>A. nodosus</i>	Ambient collection	CL	nd to 76	Knutzen and Sorland, 1982
	<i>C. rubra</i>	Ambient collection	CL	14	Knutzen and Sorland, 1982
	<i>F. serratus</i>	Ambient collection	CL	11 to 115	Knutzen and Sorland, 1982
	<i>F. vesiculosus</i>	Ambient collection	CL	5 to 166	Knutzen and Sorland, 1982
	<i>L. saccharina</i>	Ambient collection	CL	11	Knutzen and Sorland, 1982
	<i>A. nodosus</i>	Ambient collection	CL	15 to 900	Knutzen and Sorland, 1982
Fluoranthene	<i>C. rubra</i>	Ambient collection	CL	32	Knutzen and Sorland, 1982
	<i>F. serratus</i>	Ambient collection	CL	44 to 236	Knutzen and Sorland, 1982
	<i>F. vesiculosus</i>	Ambient collection	CL	40 to 412	Knutzen and Sorland, 1982
	<i>L. saccharina</i>	Ambient collection	CL	45	Knutzen and Sorland, 1982

Table 2.3.1: Observed Tissue Concentrations of PAHs in Algae as a Result of Ambient Environmental Exposure (cont'd)

Chemical	Test Organism	Life Stage or Site	Method	Tissue Concentration (ug/kg)	Reference
Fluoranthene (Cont'd)	<i>S. acutus</i>	Ambient collection	CL	91.2 to 444	Fayer et al. 1975
Indeno[1,2,3- <i>cd</i>]pyrene	<i>S. acutus</i>	Ambient collection	CL	2.36 to 50.9	Fayer et al. 1975
Methylphenanthrenes/methylantracenes	<i>A. nodosus</i>	Ambient collection	CL	75 to 323	Enutren and Sortland, 1982
	<i>C. rubrum</i>	Ambient collection	CL	52	Enutren and Sortland, 1982
	<i>F. serratus</i>	Ambient collection	CL	136 to 284	Enutren and Sortland, 1982
	<i>F. vesiculosus</i>	Ambient collection	CL	trace to 322	Enutren and Sortland, 1982
	<i>L. saccharina</i>	Ambient collection	CL	1365	Enutren and Sortland, 1982
Naphthalene/methylnaphthalene	<i>A. nodosus</i>	Ambient collection	CL	trace to 273	Enutren and Sortland, 1982
	<i>C. rubrum</i>	Ambient collection	CL	trace	Enutren and Sortland, 1982
	<i>F. serratus</i>	Ambient collection	CL	trace to 1237	Enutren and Sortland, 1982
	<i>F. vesiculosus</i>	Ambient collection	CL	trace to 2109	Enutren and Sortland, 1982

Table 2.3.3: Observed Tissue Concentrations of PAHs in Algae as a Result of Ambient Environmental Exposure (cont'd)

Chemical	Test Organism	Life Stage or Size	Method	Tissue Concentration ($\mu\text{g/g}^w$)	Reference
Naphthalene/acetylophthalene (Cont'd)	<i>L. saccharina</i>	Ambient collection	CL	166	Eutizen and Sortland, 1982
	<i>A. nodosus</i>	Ambient collection	CL	45 to 471	Eutizen and Sortland, 1982
	<i>C. rubra</i>	Ambient collection	CL	34	Eutizen and Sortland, 1982
	<i>F. serratus</i>	Ambient collection	CL	34 to 146	Eutizen and Sortland, 1982
Phenanthrene	<i>L. saccharina</i>	Ambient collection	CL	67	Eutizen and Sortland, 1982
	<i>A. nodosus</i>	Ambient collection	CL	15 to 388	Eutizen and Sortland, 1982
	<i>C. rubra</i>	Ambient collection	CL	20	Eutizen and Sortland, 1982
	<i>F. serratus</i>	Ambient collection	CL	44 to 224	Eutizen and Sortland, 1982
Pyrene	<i>L. saccharina</i>	Ambient collection	CL	31 to 325	Eutizen and Sortland, 1982
	<i>A. nodosus</i>	Ambient collection	CL	34 to 146	Eutizen and Sortland, 1982
	<i>C. rubra</i>	Ambient collection	CL	20	Eutizen and Sortland, 1982
	<i>F. serratus</i>	Ambient collection	CL	28 to 286	Eutizen and Sortland, 1982

Table 2.3.3: Observed Tissue Concentrations of PAHs in Algae as a Result of Ambient Environmental Exposure (cont'd)

Chemical	Test Organism	Life Stage or Size	Method	Tissue Concentration ($\mu\text{g/g}^{\text{dw}}$)	Reference
	<i>L. saccharina</i>	Ambient collection	CL	36	Gnutzen and Soriland, 1982

Footnotes: a - CL = cells in liquid culture
b - nd = non-detectable
c - 1g dry weight

These data are not too useful for regulatory processes, except as an indication of the variability in bioaccumulation of a particular PAH among algal species. This indicates that if particular species are to be chosen as biomonitors for PAH, either a broad range (to establish total ecosystem accumulation) or a well defined narrow range (to establish worst case) should be chosen according to the objective of the survey. The lack of data which relate dose to tissue concentration hinders the understanding of how a particular concentration in the ambient environment may impact biological processes.

The primary effort towards structure-activity relationships for PAH have focused on algae, most likely due to the ease with which algae may be exposed to PAH and the effects assessed. Casserly et al. (1983) assessed bioconcentration of various organics by algal cells in relation to the octanol-water partition coefficient (K_{ow}). The PAH studied were: naphthalene, phenanthrene and pyrene; there were several other organic compounds included in the study. The bioconcentration factors (BCF) for the PAH (4.10, 4.38, and 4.56 respectively) were at the higher end of the observed range. The relationship between $\log_{10}K_{ow}$ was determined to be linear, with the following form: $\log_{10}BCF = 0.46\log_{10} K_{ow} + 2.36(r^2 = .83)$. This study also exposed the algae to the three PAH together (along with the other compounds in the study) and demonstrated that the BCF's were not different from those obtained from single compound studies. This is a very small piece of evidence that the assumption of additivity may be appropriate in assessing the ecological impact of PAH mixtures from single compound studies.

A study by Mailhot (1987) examined ten physicochemical properties (including K_{ow}) for their relationships to algal bioconcentration and uptake rate of nine organic compounds including anthracene. For the five most lipophilic compounds (including anthracene), capacity factor (k') and $\log_{10}K_{ow}$ best predicted the bioconcentration factor (BCF) for the algae. The regression relationship for $\log_{10}K_{ow}$ is very close to

that determined in the previously described study:

$$\log_{10} \text{BCF} = 2.6 + 0.28 \log_{10} K_{ow} \quad (r^2 = 0.64).$$

The equation relating the capacity factor to BCF was:

$$\log_{10} \text{BCF} = 3.1 + 0.74 \log_{10} k' \quad (r^2 = 0.68).$$

When these relationships are extended to include hydrophilic compounds, more generalized models result. A plot of the data suggests however, that the hydrophilic compounds segregate themselves away from the regression line and the lipophilic compounds.

2.3.2.2.2 Algal Metabolism of PAH

The experimental studies on algal PAH metabolism focus mainly on benzo[a]pyrene, fluoranthene, naphthalene, phenanthrene, and pyrene (Table 2.3.4). The studies in this section were primary or very good secondary, with the exception of Kirso et al. (1983) in which the exposure duration was uncertain. Algal cells contribute to the metabolism of PAH and they function as photosensitizers for the photolytic degradation of PAH. There is also evidence that PAH may be synthesized by algae. No growth reduction or injury to the cells is reported in the studies in this section. Although injury may have been occurring, the experimental concentrations for most of these studies were lower than those described in section 2.3.2.1, where concentrations resulting in toxicity were investigated. The primary metabolites of B[a]P in *Selenastrum capricornutum* were 4,5-dihydroxy-4,5-dihydro B[a]P, and 7,8-dihydroxy-7,8-dihydro B[a]P (Lindquist and Warshawsky 1985) although between 66 and 90% of the B[a]P may not have been broken down (Warshawsky et al. 1983). In general, these metabolites were found in the medium surrounding the cells (Warshawsky et al. 1983). This suggests that B[a]P, at least, is taken up by the cells, and the metabolites are excreted. This sequence of events has not been confirmed for other species of algae or chemicals.

Table 2.3.4: Algal Metabolism of PAHs

Chemical	Test Organism	Life Stage or Size	Method	Response Variable	Effect	Exposure Concentration ($\mu\text{g/L}$)	Exposure Duration	Reference
Benz[a]anthracene	<i>Chlorella vulgaris</i>	Active culture	CL L	^{14}C labelled PAH	13.6 fold increase	n/a ^a	19 days	Bornhoff et al., 1986b
Benz[b]fluoranthene	<i>C. vulgaris</i>	Active culture	CL L	^{14}C labelled PAH	11.1 fold increase	n/a	19 days	Bornhoff et al., 1986b
Benz[k]fluoranthene	<i>C. vulgaris</i>	Active culture	CL L	^{14}C labelled PAH	7.2 fold increase	n/a	19 days	Bornhoff et al., 1986b
Benz[ghi]perylene	<i>C. vulgaris</i>	Active culture	CL L	^{14}C labelled PAH	16.4 fold increase	n/a	19 days	Bornhoff et al., 1986b
Benz[a]pyrene	<i>Chara acutirostris</i>	Active culture	CL L	Chemical degradation	5 fold, in presence of algae	2.02×10^{-4} to 4.97×10^{-4}	5 hours(?)	Bornhoff et al., 1986b
	<i>C. vulgaris</i>	Active culture	CL	^{14}C labelled PAH	10.7 fold increase	n/a	19 days	Bornhoff et al., 1986b
	<i>Cladophora glomerata</i>	Active culture	CL	Chemical degradation	5.7 fold, in presence of algae	2.02×10^{-4} to 4.97×10^{-4}	8 hours(?)	Bornhoff et al., 1986b
	<i>Enteromorpha intestinalis</i>	Active culture	CL	Chemical degradation	8.5 fold, in presence of algae	2.02×10^{-4} to 4.97×10^{-4}	8 hours(?)	Bornhoff et al., 1986b
	<i>Fucus vesiculosus</i>	Active culture	CL	Chemical degradation	no effect	2.02×10^{-4} to 4.97×10^{-4}	8 hours(?)	Bornhoff et al., 1986b
	<i>Selenastrum capricornutum</i>	Active culture	CL L	Chemical degradation	(3% degraded to cis-dihydrodiols)	1.2	4 days	Lindquist and Warshawsky, 1985
	<i>S. capricornutum</i>	Active culture	CL	Chemical degradation	metabolites mostly dihydrodiols	1.21	4 days	Zeman, et al., 1984
	<i>S. capricornutum</i>	Active culture	CL L	Chemical degradation	most B[a]P residues (86-91%); major metabolites are diols	0.160 to 1.20	4 days	Warshawsky et al., 1983

Table 2.3.4: Algal Metabolism of PAHs (cont'd)

Chemical	Test Organism	Life Stage or Size	Method	Response Variable	Effect	Exposure Concentration (mg/L)	Exposure Duration	Reference
Fluoranthene	<i>A. cylindrica</i>	Active culture	CL	Chemical degradation	11 fold. in presence of algae	4.04	3 to 4 hours	Zepp and Schlottbauer, 1983
	<i>Chlorella</i> sp.	Active culture	CL	Chemical degradation	6.5 fold. in presence of algae	4.04	3 to 4 hours	Zepp and Schlottbauer, 1983
	<i>C. vulgaris</i>	Active culture	CL	¹⁴ C labelled PAH	15.7 fold increase	n/a	15 days	Borneff et al., 1988b
	<i>C. bryopsis</i>	Active culture	CL	Chemical degradation	7.5 fold. in presence of algae	4.04	3 to 4 hours	Zepp and Schlottbauer, 1983
	<i>Chlorella</i> sp. 1	Active culture	CL	Chemical degradation	10 fold. in presence of algae	4.04	3 to 4 hours	Zepp and Schlottbauer, 1983
Indeno[1,2,3-cd]pyrene	<i>Chlorella</i> sp. 2	Active culture	CL	Chemical degradation	7.3 fold. in presence of algae	4.04	3 to 4 hours	Zepp and Schlottbauer, 1983
	<i>S. capricornutum</i>	Active culture	CL	Chemical degradation	no effect	4.04	3 to 4 hours	Zepp and Schlottbauer, 1983
	<i>C. vulgaris</i>	Active culture	CL	¹⁴ C labelled PAH	13.3 fold increase	n/a	19 days	Borneff et al., 1988b
Naphthalene	<i>A. cylindrica</i>	Active culture	CL	Chemical degradation	1.3 fold. in presence of algae	2.56	3 to 4 hours	Zepp and Schlottbauer, 1983
	<i>Chlorella</i> sp.	Active culture	CL	Chemical degradation	2.7 fold. in presence of algae	2.56	3 to 4 hours	Zepp and Schlottbauer, 1983
	<i>C. bryopsis</i>	Active culture	CL	Chemical degradation	2.2 fold. in presence of algae	2.56	3 to 4 hours	Zepp and Schlottbauer, 1983
	<i>Chlorella</i> sp. 1	Active culture	CL	Chemical degradation	2.0 fold. in presence of algae	2.56	3 to 4 hours	Zepp and Schlottbauer, 1983

Table 2.3.4: Algal Metabolism of PAHs (cont'd)

Chemical	Test Organism	Life Stage or Size	Method	Response Variable	Effect	Exposure Concentration $\mu\text{g/L}$	Exposure Duration	Reference
Phenanthrene	<i>Chlorogonium</i> sp. 2	Active culture	CL	Chemical degradation	no effect	2.56	3 to 4 hours	Zepp and Schlottbauer, 1983
	<i>S. capricornutum</i>	Active culture	CL	Chemical degradation	2.2 fold. in presence of algae	2.56	3 to 4 hours	Zepp and Schlottbauer, 1983
	<i>A. cylindrica</i>	Active culture	CL	Chemical degradation	0.15 fold. in presence of algae	3.56	3 to 4 hours	Zepp and Schlottbauer, 1983
	<i>Chlorella</i> sp.	Active culture	CL	Chemical degradation	0.57 fold. in presence of algae	3.56	3 to 4 hours	Zepp and Schlottbauer, 1983
	<i>C. bryopsis</i>	Active culture	CL	Chemical degradation	0.17 fold. in presence of algae	3.56	3 to 4 hours	Zepp and Schlottbauer, 1983
	<i>Chlorogonium</i> sp. 1	Active culture	CL	Chemical degradation	0.51 fold. in presence of algae	3.56	3 to 4 hours	Zepp and Schlottbauer, 1983
Pyrene	<i>Chlorogonium</i> sp. 2	Active culture	CL	Chemical degradation	0.24 fold. in presence of algae	3.56	3 to 4 hours	Zepp and Schlottbauer, 1983
	<i>S. capricornutum</i>	Active culture	CL	Chemical degradation	2.4 fold. in presence of algae	3.56	3 to 4 hours	Zepp and Schlottbauer, 1983
	<i>A. cylindrica</i>	Active culture	CL	Chemical degradation	0.84 fold. in presence of algae	4.04	3 to 4 hours	Zepp and Schlottbauer, 1983
	<i>Chlorella</i> sp.	Active culture	CL	Chemical degradation	1.8 fold. in presence of algae	4.04	3 to 4 hours	Zepp and Schlottbauer, 1983
	<i>C. bryopsis</i>	Active culture	CL	Chemical degradation	2.2 fold. in presence of algae	4.04	3 to 4 hours	Zepp and Schlottbauer, 1983

Table 2.3.4: Algal Metabolism of PAHs (cont'd)

Chemical	Test Organism	Life Stage or Size	Method	Response Variable	Effect	Exposure Concentration (µg/L)	Exposure Duration	Reference
Chemical	Chlorogonium sp. 1	Active culture	CL	Chemical degradation	1.2 fold, in presence of algae	4.04	3 to 4 hours	Zepp and Schlottbauer, 1983
	Chlorogonium sp. 2	Active culture	CL	Chemical degradation	no effect	4.04	3 to 4 hours	Zepp and Schlottbauer, 1983
	S. cypricornutum	Active culture	CL	Chemical degradation	1.1 fold, in presence of algae	4.04	3 to 4 hours	Zepp and Schlottbauer, 1983

Footnotes: a - CL, L : cells in liquid culture; radiolabel
 b n/a - not applicable: this study did not expose plants to PAH
 c - duration somewhat uncertain

The process of PAH metabolization is dependent to some extent on the quality of light to which the algal cells are being exposed (Warshawsky et al. 1983). Growth of S. capricornutum was completely inhibited by 0.160 mg/L B[a]P, when grown under cool white light. When grown under gold light, the growth of this algae was not affected by concentrations of B[a]P up to and including 12 mg/L. The difference between these two light sources is that white light has far more radiation at 400 nm, the upper limit for photodegradation of B[a]P, than does gold light. It is not clear how the white light enhances the photo-induced toxicity of B[a]P, but the distribution of metabolites in the medium following illumination is light quality specific. This suggests that the metabolites of B[a]P degradation are phytotoxic, and that the phytotoxicity of the metabolite pool is dependent upon the light source.

The interaction of light quality and PAH toxicity has been further investigated (Cody et al. 1984), using three types of light (gold, black and cool white). This study was similar to the work of Warshawsky et al. (1983), and indicated that S. capricornutum was more sensitive to B[a]P under black light than cool white, probably due to the greater quantity of energy ≤ 400 nm in black light. This study also indicated that out of 13 metabolites of B[a]P, five were toxic to the algae under white light; none were toxic under gold light. These compounds were, in order of decreasing toxicity, 3,6 quinone, 6 phenol, 9 phenol and 3 phenol, and 1,6 quinone of B[a]P. The proposed mechanism of toxicity is the formation of PAH-DNA adducts, leading to membrane damage or cell death. Alternatively, the formation of radicals (hydroxyl, semi-quinone) as a result of an oxidation-reduction cycle involving quinone, hydroquinone and molecular oxygen, may be cytotoxic.

The presence of many algal species (Zepp and Schlotzhauer 1983, Kirso et al. 1983) increased the rate of degradation of several PAH (B[a]P, fluoranthene and naphthalene). The degradation of phenanthrene was in general slower in the presence of algae, with the exception of

S. capricornutum. The degradation of pyrene was generally faster in the presence of algae, except for A. cylindrica. It should be noted that the majority of these studies were carried out at 2.56 mg/L, a concentration of naphthalene at which one might expect loss of nitrogen fixation capacity (Bastian and Toetz 1985). The authors (Zepp and Schlotzhauer 1983) concluded that the algal-induced degradation involved the reversible binding of the PAH to the algal cell, where compounds exist which increase the sensitivity of the PAH to light. However, when the experimental rate of degradation is combined with the population density of algae typical in the ambient environment, the authors conclude that algal mediated photosensitization would play a very small role in reducing PAH concentration in the aquatic environment. It is likely, however, that the rate would be higher for compounds more hydrophobic than the ones examined here (pyrene, phenanthrene, fluoranthene, naphthalene).

There is evidence that PAH load to the environment may not be totally anthropogenic. Algal cells supplied with ^{14}C acetate as the sole carbon source contained similar quantities of seven PAH as those on non-labeled substrate, but the PAH from the ^{14}C supplied algae contained between seven and 16 times as much label as control cultures (Borneff et al. 1968a,b). This suggests that algal cells may be capable of de novo synthesis of many PAH. The authors suggest that the endogenous PAH may act as growth regulators, in a manner analogous to carcinogenicity in animals. The ability of some PAH to stimulate cell growth has been observed in laminar algae, and has been discussed in section 2.3.2.1. Hutchinson et al. (1980) determined the relationship of various physicochemical properties to the algal toxicity of a group of hydrocarbons. The PAH included in the list of 38 compounds were: naphthalene, 1-methyl-, and 2-methyl-naphthalene, phenanthrene, anthracene and pyrene. These compounds are of similar molecular weight, but cover a range of solubilities and K_{ow} 's. Toxicity was reported as an EC50, the PAH concentration which reduced photosynthesis to 50% that of controls. It should be noted that the EC50's for anthracene and pyrene were extrapolated, as

the concentrations required to reduce photosynthesis by 50% were in excess of the solubility of these compounds in water. For these algae, the EC50 was directly related to water solubility, and inversely related to $\log_{10}K_{ow}$: $\log_{10}EC50 = -0.101 + 0.826\log_{10}S$ and $\log_{10}EC50 = 5.176 - 0.928\log_{10}K_{ow}$. These results are consistent with Mailhot (1987) and Casserly et al. (1983) which suggested that in the case of algae, lipophilic compounds are likely to be more toxic due to their greater affinity for plant cell walls and membranes, relative to their aqueous surroundings. This relationship is likely to be similar for higher plants, as lipophilic compounds would demonstrate less affinity for the aqueous transpiration stream than for cell membranes. Unfortunately, there is no published reference which describes this sort of relationship for PAH bioaccumulation alone.

2.4 MODIFYING FACTORS OF TOXICITY

A variety of factors influence the aquatic toxicity of PAH to fish, invertebrates and plants. Modifying factors have classically been categorized according to their origin as either abiotic (e.g. chemical, physical factors) or biotic, factors intimately associated with the biology of an organism (e.g. sex, age, route of uptake). Since several factors may influence the toxicity of a compound to a diverse group of organisms, the present discussion reflects this categorical approach, with the intent of addressing the most pertinent factors from a regulatory perspective.

2.4.1 Abiotic Modifying Factors

2.4.1.1 Photoinduced Toxicity

A conventional view of the acute toxicity of PAH has been that such hazards are less pertinent than the potential for carcinogenic and mutagenic effects. This has arisen because of the limited toxicity demonstrated by PAH compounds when tested under visible light and within the confines of their aqueous solubility. However, increasing evidence suggests that numerous PAH compounds are more acutely toxic to aquatic invertebrates and fish than previously documented as a result of photoactivation.

Photoinduced toxicity is important for three reasons. First, it renders PAH toxic at concentrations and exposure times much less than experienced in standard bioassays (See Tables 2.1.1, 2.1.2, and 2.2.2). Second, it creates a toxicity hazard from a number of high molecular weight compounds previously thought to be non-toxic. Third, it introduces another modifying factor in the design and assessment of acute bioassays.

The phenomenon is typically referred to as "photoinduced" toxicity because many of the tested compounds exhibit acute toxicity only in combination with solar or simulated solar ultraviolet radiation (SUVR). Because of the highly conjugated structure of PAH, these compounds absorb electromagnetic radiation at longer UV wavelengths than is typical for many organic compounds (Finlayson-Pitts and Pitts 1986). This so-called redshift in the absorption spectrum results in photoactivation (actinism) over wavelengths ranging from 290 nm to the short wavelengths of the visible spectrum, approximately 400 nm.

For the purpose of photochemical considerations, ultraviolet light can be divided into three regions: UV-A, 390-315 nm, UV-B, 315-285 nm and UV-C, 285 nm and lower (Landrum et al. 1986). Although much incident SUVR is filtered out by the atmosphere, it is primarily the UV-C component which is removed, therefore, the relevant SUVR components (i.e. UV-A and UV-B) remain available to evoke photoactivation of PAH in surface waters. Oris (1985) compiled a list of selected PAH which described their absorption characteristics (Table 2.4.1). The majority of these compounds exhibit absorption maxima at wavelengths which are customarily not present in conventional cool fluorescent lighting typically used in laboratories. Previous studies conducted under such circumstances, perhaps to avoid photo-oxidation of the parent compounds, therefore, may be of limited value when extrapolating the environmental hazards.

2.4.1.1.1 Toxic Mechanism

The toxic mechanism of photoactivated PAH has not been unequivocally demonstrated although free radical generation is the most plausible explanation. The available evidence suggests the following. Upon absorbing the appropriate quanta of light energy, the electron configuration of the activated PAH is transformed to the excited "triplet" state, a condition in which the outer paired electrons have identical rather than opposing spins. Transfer of the PAH excited

TABLE 2.4.1
SOLAR RADIATION ABSORPTION CHARACTERISTICS OF
SELECTED POLYCYCLIC ORGANIC COMPOUNDS (WEAST 1972;
JACOB *et al.* 1984). "*" INDICATES A LOG MOLAR
EXTINCTION COEFFICIENT GREATER THAN 3.0

Compound	<u>Absorption in Atmospheric Solar Range</u>		
	UV-B (285-345 nm)	UV-A (345-400 nm)	Visible (400-700 nm)
Acridine	*	*	-
Anthracene	*	*	-
Anthanthrene	*	*	*
Benz[a]acridine	*	*	-
Benzanthrone	*	*	*
Benz[a]anthracene	*	*	-
Benz[b]anthracene	-	*	*
Benzo[b]chrysene	*	*	-
Benzo[a]fluoranthene	*	*	*
1H-Benzo[c]fluorene	*	-	-
Benzo[g,h,i]perylene	*	*	*
Benzo[a]pyrene	*	*	*
Benzo[e]pyrene	*	*	-
Benzo[b]triphenylene	*	*	-
Chrysene	*	*	-
Coronene	*	-	-
Dibenz[a,j]acridine	*	*	-
Dibenz[a,h]anthracene	*	*	-
Fluoranthene	*	*	-
Fluorene	*	-	-
Napthalene	*	-	-
Perylene	*	*	*
Phenanthrene	-	-	-
Pyrene	*	*	-

(From Oris 1985).

state to ground state oxygen forms the singlet oxygen (super oxide free radical) which is very reactive. Because of the lipophilic nature of PAH, the majority of singlet oxygen molecules are thought to be generated in non-polar hydrophobic locations (e.g. membranes), and therefore, enhance the likelihood of further interaction with biomolecules rather than water. This may also give rise to oxidation products of PAH, hydrogen peroxide, hydroxyl radicals and other free radicals.

These reactive species lead to lipid peroxidation causing membrane damage. In the case of anthracene and SUVR-exposed bluegill sunfish, histological examination and overt signs of distress suggest that mortalities arise through a combination of respiratory and osmoregulatory distress due to damage incurred at the gill epithelium and the body surface epidermis (Oris and Giesy 1985). As a result of this mechanism, there is a requirement for both the presence of the PAH in vivo with simultaneous SUVR exposure. Toxic effects do not appear to arise from exposure to external photo-oxidized products (Bowling et al. 1983).

2.4.1.1.2 Scope of Effect

The extent to which photoactivation may modify toxicity can be quite impressive. For example, Oris and Giesy (1987), using fathead minnow larvae, conducted lethal bioassays with twelve PAH both in the dark (to circumvent photoinduced toxicity) and under simulated SUVR (see Table 2.4.2 for compound names). While none of the PAH were rapidly lethal in the dark, five of the compounds were toxic under simulated SUVR at concentrations ranging from 0.002 to 0.05 mg/L and a sixth at 0.53 mg/L (see Table 2.1.2 for individual UV-LT50's).

TABLE 2.4.2
CHEMICALS AND ORGANISMS IN WHICH PHOTOINDUCED TOXICITY
(MEDIATED THROUGH SUNLIGHT OR SIMULATED SOLAR
ULTRAVIOLET RADIATION) WAS CONFIRMED TO
BE EITHER PRESENT OR ABSENT

Chemical	Test Organism	Phototoxic Activity	Reference
Acridine	Cladoceran <u>Daphnia magna</u>	Present	Newsted & Giesy 1987
	Fathead Minnow <u>Pimephales promelas</u>	Present	Oris & Giesy 1987
Anthracene	Cladoceran <u>Daphnia magna</u>	Present	Newsted & Giesy 1987 Kagan <u>et al.</u> 1985
	Cladoceran <u>Daphnia magna</u>	Present	Oris <u>et al.</u> 1984
	Cladoceran <u>Daphnia pulex</u>	Present	Allred & Giesy 1985
	Mosquito Larvae <u>Aedes aegypti</u>	Present	Oris & Giesy 1984 Kagan <u>et al.</u> 1985
	Leopard Frog Larvae <u>Rana pipiens</u>	Present	Kagan <u>et al.</u> 1984, 1985
	Bluegill Sunfish <u>Lepomis macrochirus</u>	Present	Bowling <u>et al.</u> 1983
Anthracene		Present	Oris & Giesy 1984
		Present	Oris <u>et al.</u> 1985
	Fathead Minnow <u>Pimephales promelas</u>	Present	Oris & Giesy 1987
	Green Algae <u>Chlorella pyrenoidosa</u>	Absent	Oris <u>et al.</u> 1984
Benz[a]an- thracene	Cladoceran <u>Daphnia magna</u>	Present	Newsted & Giesy 1987
	Fathead Minnow <u>Pimephales promelas</u>	Present	Oris & Giesy 1987

TABLE 2.4.2 (cont'd)
CHEMICALS AND ORGANISMS IN WHICH PHOTOINDUCED TOXICITY
(MEDIATED THROUGH SUNLIGHT OR SIMULATED SOLAR
ULTRAVIOLET RADIATION) WAS CONFIRMED TO
BE EITHER PRESENT OR ABSENT

Chemical	Test Organism	Phototoxic Activity	Reference
Benz[b] anthracene	Cladoceran <u>Daphnia magna</u>	Present	Newsted & Giesy 1987
	Fathead Minnow <u>Pimephales promelas</u>	Marginal	Oris & Giesy 1987
Dibenz[ah] anthracene	Cladoceran <u>Daphnia magna</u>	Present	Newsted & Giesy 1987
	Fathead Minnow <u>Pimephales promelas</u>	Absent	Oris & Giesy 1987
Benzanthrone	Cladoceran <u>Daphnia magna</u>	Present	Newsted & Giesy 1987
	Fathead Minnow <u>Pimephales promelas</u>	Present	Oris & Giesy 1987
Carbazole	Cladoceran <u>Daphnia magna</u>	Absent	Newsted & Giesy 1987
Chrysene	Cladoceran <u>Daphnia magna</u>	Present	Newsted & Giesy 1987
Benzo[a] fluorene	Cladoceran <u>Daphnia magna</u>	Present	Newsted & Giesy 1987
Benzo[b] fluorene	Cladoceran <u>Daphnia magna</u>	Present	Newsted & Giesy 1987
Fluoranthene	Cladoceran <u>Daphnia magna</u>	Present	Newsted & Giesy 1987
		Present	Kagan <u>et al.</u> 1985
	Mosquito Larvae <u>Aedes aegypti</u>	Present	Kagan <u>et al.</u> 1985
	Leopard Frog Larvae <u>Rana pipiens</u>	Present	Kagan <u>et al.</u> 1985

TABLE 2.4.2 (cont'd)
CHEMICALS AND ORGANISMS IN WHICH PHOTOINDUCED TOXICITY
(MEDIATED THROUGH SUNLIGHT OR SIMULATED SOLAR
ULTRAVIOLET RADIATION) WAS CONFIRMED TO
BE EITHER PRESENT OR ABSENT

Chemical	Test Organism	Phototoxic Activity	Reference
Fluoranthene (Cont'd)	Fathead Minnow <u>Pimephales promelas</u>	Present	Kagan <u>et al.</u> 1985
Fluorene	Cladoceran <u>Daphnia magna</u>	Absent	Newsted & Giesy 1987
Perylene	Cladoceran <u>Daphnia magna</u>	Present	Newsted & Giesy 1987
	Fathead Minnow <u>Pimephales promelas</u>	Absent	Oris & Giesy 1987
Benzo[ghi] perylene	Cladoceran <u>Daphnia magna</u>	Present	Newsted & Giesy 1987
	Fathead Minnow <u>Pimephales promelas</u>	Marginal	Oris & Giesy 1987
Phenanthrene	Cladoceran <u>Daphnia magna</u>	Absent	Newsted & Giesy 1987
	Fathead Minnow <u>Pimephales promelas</u>	Absent	Oris & Giesy 1987
Pyrene	Cladoceran <u>Daphnia magna</u>	Present	Newsted & Giesy 1987
		Present	Kagan <u>et al.</u> 1985
	Mosquito Larvae <u>Aedes aegypti</u>	Present	Kagan <u>et al.</u> 1985
	Leopard Frog Larvae <u>Rana pipiens</u>	Present	Kagan <u>et al.</u> 1985
	Fathead Minnow <u>Pimephales promelas</u>	Present	Kagan <u>et al.</u> 1985
		Present	Oris & Giesy 1987
Benzo[a]pyrene	Green Algae <u>Selenastrum</u> <u>capricornutum</u>	Present	Cody <u>et al.</u> 1984

TABLE 2.4.2 (cont'd)
CHEMICALS AND ORGANISMS IN WHICH PHOTOINDUCED TOXICITY
(MEDIATED THROUGH SUNLIGHT OR SIMULATED SOLAR
ULTRAVIOLET RADIATION) WAS CONFIRMED TO
BE EITHER PRESENT OR ABSENT

Chemical	Test Organism	Phototoxic Activity	Reference
Benzo[a]pyrene (Cont'd)	Cladoceran <u>Daphnia magna</u>	Present	Newsted & Giesy 1987
		Present	Kagan <i>et al.</i> 1985
	Mosquito Larvae <u>Aedes aegypti</u>	Present	Kagan <i>et al.</i> 1985
Benzo[a]pyrene	Fathead Minnow <u>Pimephales promelas</u>	Present	Kagan <i>et al.</i> 1985
		Present	Oris & Giesy 1987
Benzo[e]pyrene	Fathead Minnow <u>Pimephales promelas</u>	Absent	Oris & Giesy 1987
Triphenylene	Cladoceran <u>Daphnia magna</u>	Absent	Newsted & Giesy 1987

A similar study addressing photoinduced toxicity towards the cladoceran, D. magna, was reported by Newsted and Giesy (1987). Their results also demonstrated that many of the otherwise non-acutely lethal PAH were rapidly lethal to D. magna at concentrations less than 0.010 mg/L (see Table 2.2.2 for UV-LT50's).

The actinic effects of PAH compounds are not unique to fish or cladocerans alone. Acute lethality from phototoxic PAH have been reported in a variety of organisms including mosquito and frog larvae (Oris et al. 1984, Kagan et al. 1984, 1985) and green algae (Cody et al. 1984). Table 2.4.2 provides a summary of tested compounds and organisms for selected studies of PAH photoinduced toxicity, data on lethal effects have already been presented in sections 2.1.1 and 2.2.1.

Phototoxic effects are less clearly defined for algae. Oris et al. (1984) reported ^{14}C bicarbonate fixation by the freshwater green alga Chlorella pyrenoidosa was not adversely affected by anthracene in the presence of SUVR. However, growth of Selenastrum capricornutum was reportedly more sensitive to PAH when a UV light source (black fluorescent bulb) was used versus a visible light source (cool-white fluorescence) (Cody et al. 1984).

Landrum et al. (1986) notes that phytoplankton which contain carotenoid pigments should be less sensitive to PAH photoinduced toxicity than fish and invertebrates due to the protective effects of beta-carotene as a singlet oxygen quencher. Currently, however, the photoinducible toxic hazard of PAH to phytoplankton warrants further resolution.

Photoinduced PAH toxicity is clearly the result of a complex series of events which is dependent upon the integration of a) tissue content of PAH in an organism; b) the amount of SUVR penetrating the aquatic environment and ultimately the organism; c) the amount of SUVR absorbed by the PAH in vivo (molar absorptivity); d) the efficiency of conversion of ground state molecules to the excited triplet state

(quantum yield); e) the probability of the excited intermediate reacting with a target molecule (Newsted and Giesy 1987). While only a fraction of the potentially phototoxic PAH compounds have actually been tested, sufficiently consistent toxicity databases have been developed to enable first attempts at modeling quantitative structure-activity relationships (Newsted and Giesy 1987, Oris and Giesy 1987).

The largest phototoxicity database for PAH is reported by Newsted and Giesy (1987) for the cladoceran D. magna and consists of twenty compounds (see Table 2.2.2 for toxicity data). They investigated seven descriptors which included: lowest-energy singlet state (ES, kJ/mol), lowest energy triplet state (ET, kJ/mol), the difference between lowest singlet and lowest triplet states (singlet-triplet splitting energy, EST, kJ/mol), the phosphorescence lifetime (TP, s), first and second-order molecular connectivity indices, and log octanol/water partition coefficient (K_{ow}).

After normalizing LT50's to a unified PAH body concentration of 77.19 nM/g wet weight of D. magna, it was determined that both ET and TP provided the highest correlations with toxicity. That ET was highly correlated suggests that formation of the triplet energy state is an integral part of the phototoxic mechanism as described earlier. The relationship with phosphorescence lifetime (TP) was intriguing, in that compounds with long lifetimes (e.g. TP > 3.5 s) were not toxic. It was noted that compounds with TP values less than 2 s were extremely toxic and compounds with TP values between 2 and 3 s were moderately toxic. This suggested that compounds with long phosphorescence lifetimes were too stable to react with biomolecules and were therefore not phototoxic. A curvilinear equation using triplet energy yielded the best predictive model for the normalized LT50s (Figure 2.4.1).

Canonical discriminant analysis using phosphorescence lifetime and lowest triplet energy was used to classify the twenty tested PAH into toxic categories of very toxic (LT50 < 900 min), moderately toxic

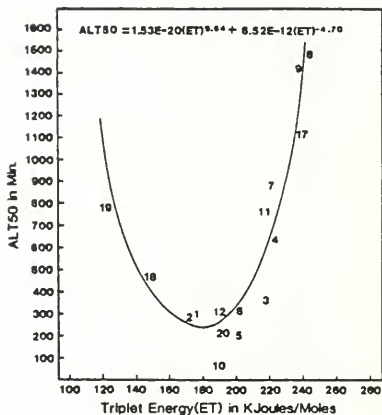
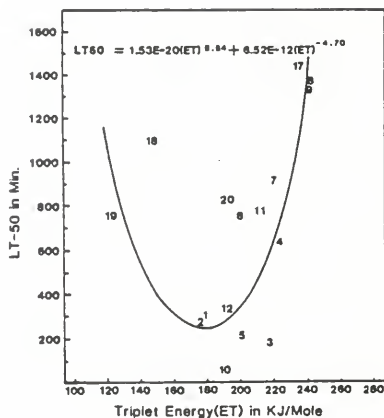
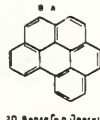
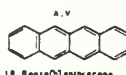
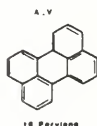
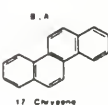
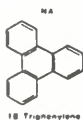
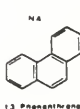
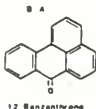
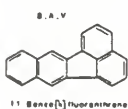
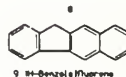
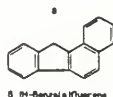
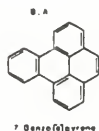
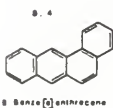
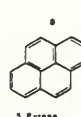
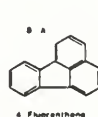
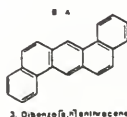
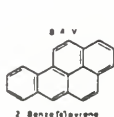
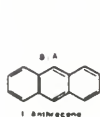


Fig. 2.4.1 LEFT: Median lethal time (LT50) as a function of lowest triplet energy (ET). RIGHT: Adjusted median lethal time (ALT50) as a function of lowest triplet energy. The polycyclic aromatic hydrocarbons are identified by number below.

(Modified from Newsted & Giesy 1987)



(900 < LT50 < 9999 min) or non-toxic. The classifications are summarized in Table 2.4.3 for the twenty tested compounds and an additional 9 compounds which were modeled but not tested. In all cases, the posterior probability was greater than 98% that the compounds were correctly classified.

It is notable that 21 of 29 compounds were categorized as "very toxic", which accentuates the need to consider PAH photoinduced toxicity when evaluating the environmental hazards of the chemicals.

Oris and Giesy (1987) undertook a similar analysis to predict PAH photoinduced toxicity towards fathead minnows, but were restricted to a more limited database of six phototoxic compounds. Linear regression was of limited value because no univariate predictive relationships were observed. Stepwise discriminant analysis using the same set of descriptors as Newsted and Giesy (1987) identified phosphorescence lifetime and the first order molecular connectivity index as the most appropriate descriptors for classifying PAH as phototoxic (LT40<96h) or non-phototoxic (96h<LT50).

The results of the classification are presented in Table 2.4.4, and notable differences exist between the classification schemes for fathead minnows and D. magna. Only four of the sixteen "nontoxic" compounds, (fluorene, carbazole, coronene and phenanthrene) are consistent with the classification of nontoxic compounds based on D. magna.

To date, only a small sample of compounds have been effectively addressed for their photoinduced toxicity, however the ability to model this early database to predict additional phototoxic compounds appears promising. For the purposes of environmental regulation, a standardized approach will be required which addresses not only the aqueous concentration of PAH but also unifies PAH exposure to a representative SUVR intensity and exposure time similar to the approach described by Oris and Giesy (1986).

TABLE 2.4.3

MODELING DESCRIPTORS AND PHOTOTOXIC CLASSIFICATION OF SELECTED
PAH BASED ON LETHALITY RESPONSE OF THE CLADOCERAN, *Daphnia magna*

Compound	TP ^a (s)	ET ^b (kJ/mol)	Phototoxic ^d Category
Benzanthrone	0.02	192	1
Pyrene	0.63	203	1
Acridine	0.15	190	1
Anthracene	0.09	178	1
Benzo[a]pyrene	0.11	177	1
Benz[a]anthracene	0.36	200	1
Dibenz[ah] anthracene	1.60	218	1
Benzo[e]pyrene	2.12	221	1
Perylene	3.50	148	1
Phenanthrene	2.94	259	3
Benz[b]anthracene	0.01	123	1
Benzo[k]fluoranthene	0.83	211	1
Chrysene	2.54	239	2
Fluoranthene	0.99	221	1
Fluorene	5.00	284	3
1H-Benzo[a]fluorene	2.61	241	2
1H-Benzo[b]fluorene	2.24	240	2
Carbazole	8.04	293	3
Triphenylene	15.0	282	3
Benz[ghi] perylene	0.44	193	1
Dibenz[ah] acridine ^c	2.31	218	1
Benzo[a]acridine ^c	0.41	213	1
Benzo[c]acridine ^c	0.28	213	1
Coronene ^c	9.50	232	3
Phenazine ^c	0.08	186	1
Dibenz[aj] anthracene ^c	2.51	221	1
Benzo[b]chrysene ^c	0.18	190	1
Dibenzo[ac] phenazine ^c	0.29	223	1
Benzo[b] triphenylene ^c	0.80	213	1

Footnotes: a - Phosphorescence lifetime (TP)

b - Lowest triplet energy

c - PAH compounds modeled but not empirically tested

d - Classification: 1 - very toxic (LT50<900 min),

2 - moderately toxic (900<LT50<9999 min),

3 - non-toxic

(Modified from Newsted and Giesy, 1987)

TABLE 2.4.4
MODELING DESCRIPTORS AND PHOTOTOXIC CLASSIFICATION OF SELECTED
PAH BASED ON LETHALITY RESPONSE OF THE FATHEAD MINNOW
(*Pimephales promelas*)

Compound	τ^a (s)	Phototoxic ^d MCI ^b	Category
Benz[a]anthracene	0.36	6.2201	Toxic
Benz[b]anthracene ^c	0.01	6.2141	Toxic
Phenazine ^c	0.08	4.6546	Toxic
Acridine	0.15	4.5856	Toxic
Anthracene	0.09	4.8094	Toxic
Benz[c]acridine ^c	0.28	5.8541	Toxic
Benz[a]acridine ^c	0.41	5.8541	Toxic
Benzanthrone	0.02	6.2635	Toxic
Pyrene	0.63	5.5594	Toxic
Benzo[a]pyrene	0.11	6.9701	Toxic
Benzo[b]chrysene ^c	0.18	7.6308	Toxic
Dibenz[a,c]phenazine	0.29	7.4819	Toxic
Benzo[g,h,i]perylene	0.44	7.7201	Notox
Fluoranthene ^c	0.99	5.5654	Notox
Benzo[k]fluoranthene ^c	0.83	6.9701	Notox
Benzo[b]triphenylene ^c	0.80	7.3867	Notox
1H-Benzo[a]fluorene ^c	2.61	6.0225	Notox
1H-Benzo[b]fluorene ^c	2.24	6.0166	Notox
Chrysene ^c	2.54	6.2261	Notox
Fluorene ^c	5.00	4.5118	Notox
Dibenz[a,h]acridine ^c	2.31	7.5535	Notox
Carbazole ^c	8.04	4.4046	Notox
Coronene ^c	9.50	8.2142	Notox
Dibenz[a,j]anthracene ^c	2.51	7.3421	Notox
Dibenz[a,h]anthracene	1.60	7.6308	Notox
Benzo[e]pyrene	2.12	6.9761	Notox
Phenanthrene	2.94	4.8154	Notox
Perylene	3.50	6.9761	Notox

Footnotes: a - Phosphorescence lifetime
b - First order molecular connectivity index
c - PAH compounds modeled but not empirically tested
d - Classification: Toxic - LT50<96h, Notox - LT50>96h

(Modified from Oris and Giesy, 1987)

This is illustrated by the predicted chronic and acute toxicity relationships developed for anthracene (Oris and Giesy 1986). As daily SUVR exposure is lengthened, the threshold concentrations for predicted effects are reduced (Figure 2.4.2). An additional point is that the absolute difference between chronic and acute threshold concentrations also decreases as daily SUVR exposure is increased. This suggests that the difference between predicted chronic and acute toxicity is very small at high SUVR exposures, and extreme regulatory care is required in developing appropriate safe limits.

The concept and implications of PAH photoinduced toxicity have historically received only minor consideration in the assessment of acute aquatic toxicity of these compounds. This may partially have arisen as a result of striving to achieve test conditions most conducive to maintaining the unmodified (i.e. non-photooxidized) parent compound during exposures.

Clearly further work is required to resolve the differences between the fish based classification and that of D. magna. Intuitively, one might rationalize that a more rapid uptake of PAH would occur in D. magna because of a more conducive surface area to body volume ratio. Such an effect would increase the apparent toxicity of the compound since it may reach the site of action at an earlier point during the cladoceran exposure, versus that of fish. It should be recognized that detailed studies of photoinduced toxicity in fish are limited to data derived in the fathead minnow (Pimephales promelas). These data offer limited predictive value to effects in other species.

2.4.1.2 Temperature

Studies addressing temperature as a modifying factor are summarized in Table 2.4.5. Only limited data is available regarding the influence of temperature on lethal toxicity. Smith et al. (1988) noted a significant increase in lethal toxicity of phenanthrene to Daphnia

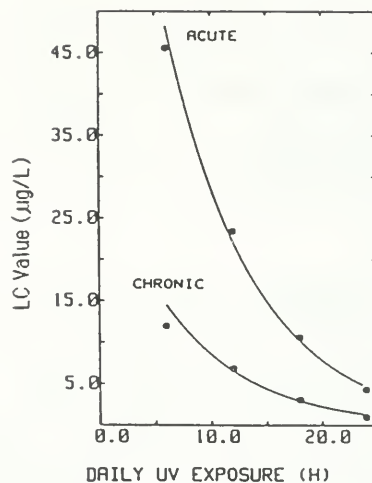


Fig. 2.4.2 Predicted thresholds for acute (96h LC50) and chronic (LCI) toxicity of anthracene as a function of daily (SUVR Exposure time).

Equation of curves: acute; $\ln(96h\ LC50) = 4.656 - 0.130\ (SUVR\ Exposure\ Time)$,
 chronic; $\ln(LCI) = 3.48 - 0.134\ (SUVR\ Exposure\ Time)$.

Source: Oris and Giesy 1986

pulex over the narrow temperature range of 17°C to 20°C, while only a marginal effect was noted for 1,3-dimethylnaphthalene. The data of Cooney and Gehrs (1984) suggest that lethal toxicity of acridine during early development of the calanoid copepod, Diaptomus clavipes, decreases with increasing temperature. However, this is more likely a function of exposure time due to the prolonged development (and hence exposure) of the egg-to-nauplius stage at low temperatures. Data for the nauplius-to-copepodid stage suggest enhanced lethal toxicity at higher temperatures. Trends towards decreased survival of mud crab larvae Rhithropanopeus harrisi at lower temperatures during exposure to naphthalene or phenanthrene were not significant (Laughlin and Neff 1979).

The major effect of temperature appears to be its influence on tissue concentrations and the ability to metabolize PAH. A decrease in environmental temperature is associated with a decreased rate of metabolic clearance of PAH (Harris et al. 1977, Collier et al. 1978, Laughlin and Neff 1979, Varanasi et al. 1981a, Fucik and Neff 1977). As a result, higher concentrations of PAH are usually observed in fish and invertebrates when exposed at lower temperatures, even though uptake kinetics may be slower (Varanasi et al. 1981a).

In addition, higher levels of metabolites are also observed in tissues of fish at low versus high temperatures (Varanasi et al. 1981a). This may arise through several possible mechanisms. First, it appears that some fish (Fundulus heteroclitus, Salmo gairdneri) adapted to lower temperatures have a higher level of background MFO activity on a per unit hepatic microsomal basis (Stegman 1979, Egaas and Varanasi 1982). Hence, there is greater enzymatic capacity to generate metabolites in uninduced fish at low temperatures. Secondly, acclimation to low temperature also appears to compromise secondary biotransformation (i.e. conjugation) of at least B[a]P. This results in an increased ratio of primary to secondary metabolites of B[a]P in rainbow trout (Egaas and Varanasi 1982), theoretically enhancing the potential for toxicity via the more reactive primary metabolites.

TABLE 2.4.5

INFLUENCE OF TEMPERATURE ON THE AQUATIC TOXICITY OF PAH COMPOUNDS

CHEMICAL	TEST ORGANISM	PROTOCOL	EFFECT	COMMENTS	REFERENCE
Acridine	Copepod <u>Diaptomus clavipes</u>	16°, 21°, 26°C (waterborne)	Lethal Toxicity	-trend towards increased toxicity at lower temperature for egg-to-nauplius stage -trend towards increased toxicity at higher temperature for nauplius-to-copepodid stage	Cooney and Gehrs 1984
Anthracene	Amphipod <u>Pontoporeia hoyi</u>	4°, 7°, 10°, 15°C (waterborne)	Uptake	-increase in uptake rate coefficient from 136 h ⁻¹ to 215 h ⁻¹ over the temperature range tested	Landrum 1982
Naphthalene	Copepods <u>Calanus eurytemora</u>	5° to 16°C (waterborne)	Depuration	retention decreased as temperature increased	Harris et al. 1977
	Clams <u>Rangia cuneata</u> & <u>Protothaca staminea</u>	15°, 20°, 25°, 30°C (waterborne)	Uptake	significantly higher body concentrations at lowest temperature	Pucik and Neff 1977
	Coho salmon <u>Oncorhynchus kisutch</u>	4°, 10°C (dietary)	Depuration	decreased temperature decreased depuration after force-feeding	Collier et al. 1978
	Mud crab <u>Rhithropanopeus harrisi</u>	20°, 25°, 30°C (waterborne)	Lethal Toxicity	trend towards increased toxicity at lower temperature, but not significant	Laughlin and Neff 1979
	Starry flounder <u>Platichthys stellatus</u>	4°, 12°C (dietary)	Depuration	-highest liver concentration at 4°C after 24 h (34 times relative to 12°C) -due to increased retention and decreased metabolism	Varanasi et al. 1981
1,3-dimethylnaphthalene	Cladoceran <u>Daphnia pulex</u>	17°, 20°C (waterborne)	Lethal Toxicity	marginal increased toxicity at higher temperature	Smith et al. 1988
Phenanthrene	Cladoceran <u>Daphnia pulex</u>	17°, 20°C (waterborne)	Lethal Toxicity	marginal increased toxicity at higher temperature	Smith et al. 1988

TABLE 2.4.5 (cont'd)

INFLUENCE OF TEMPERATURE ON THE AQUATIC TOXICITY OF PAH COMPOUNDS

CHEMICAL	TEST ORGANISM	PROTOCOL	EFFECT	COMMENTS	REFERENCE
Phenanthrene	Mud crab <u>Rhithropanopeus harrisii</u>	20°, 25°, 30°C (waterborne)	Lethal Toxicity	trend towards increased toxicity at lower temperature, but not significant	Laughlin and Neff 1979
Benzo[a]pyrene	Spiny lobster <u>Panulirus argus</u>	15°, 28° C (1 mg/kg, injection)	Depuration	-elimination half-lives were 1.11 and 2.25 wks at warm and cold temperatures, respectively -effect due to more rapid excretion rather than metabolism	Little et al. 1985
	Bluegill Sunfish <u>Lepomis macrochirus</u>	13°, 23° C (waterborne)	Uptake/Depuration	-increased temperature increased uptake six-fold but only increased elimination three-fold -biotransformation of B[a]P reduced at lower temperature	Jimenez et al. 1987
	Mummichog <u>Fundulus heteroclitus</u>	6.5°, 16.5° C (10 mg/kg, injection)	MFO Activity	-MFO induction repressed at cold temperature -uninduced MFO activity higher in cold-acclimated fish	Stegeman 1979
	Rainbow trout <u>Salmo gairdneri</u>	7°, 16° C (in vitro)	DNA Binding Metabolism MFO Activity	-DNA binding increased five-fold at 7°C relative to 16°C - <u>in vitro</u> liver AHH activity of uninduced fish increased at 7°C relative to 16°C -subsequent increase in conversion of B[a]P to metabolites which bind to DNA -greater proportion of primary vs secondary metabolites at 7° vs 16°C	Egaas and Varanasi 1978

2.4.1.3 Complexation

Complexation of PAH with dissolved organic matter (DOM) can significantly modify bioconcentration. In particular, dissolved humic material (DHM), a constituent of dissolved organic carbon (DOC), has been demonstrated to render significant fractions of certain PAH unavailable for uptake by fish and invertebrates (Spacie et al. 1983, McCarthy and Jimenez 1985, McCarthy et al. 1985). DHM represents the refractory organic matter formed by microbial decomposition and chemical polymerization of plant components, and is primarily composed of polyphenolic compounds (McCarthy et al. 1985). In most lakes and rivers, DHM may range from 0.5 to 4.0 mg C/L, although concentrations in wetlands and marshes may reach 10 to 50 mg C/L (Thurman 1984).

It is generally believed that DOM-complexed PAH are rendered unavailable to biota through two related mechanisms. First, the complexed xenobiotic is too large to facilitate transport across respiratory membranes (DHM ranges from 5,000 to 100,000 daltons, McCarthy et al. 1985). Secondly, the rate of desorption of PAH from DHM is too slow to facilitate uptake of the freely dissolved PAH, even when the complex passes in close proximity to respiratory membranes (Landrum et al. 1987).

On the basis of this mechanism, increased DHM results in increased complexation of waterborne PAH, and therefore a reduction in bioavailability results. This principle can be further extended to predict that the uptake rate coefficient, K_u , of various PAH will be reduced in the presence of DHM (McCarthy et al. 1985). While this has been observed (Table 2.4.6), Landrum et al. (1987) note that this effect is observed only if uptake kinetics are related to the total waterborne PAH concentration. If only the freely dissolved fraction is considered, K_u should remain unchanged.

TABLE 2.4.6
INFLUENCE OF COMPLEXATION ON THE BIOAVAILABILITY OF PAH TO AQUATIC ORGANISMS

CHEMICAL	TEST ORGANISM	COMPLEXING AGENT	CONCENTRATION (µg C/L)	COMMENT	REFERENCE
Naphthalene	Cladoceran <u>Daphnia magna</u>	DHM	60	no observed effect on k_u	McCarthy et al. 1985
	Bluegill Sunfish <u>Lepomis macrochirus</u>	DHM	20	no observed effect on k_u	McCarthy & Jiminez 1985
Anthracene	Cladoceran <u>Daphnia magna</u>	DHM	10, 30, 60	calculated reduction in k_u = 12%, 29% and 46% respectively	McCarthy et al. 1985
	Bluegill Sunfish <u>Lepomis macrochirus</u>	DHM	1.0	no observed effect on k_u	Spacie et al. 1983
Phenanthrene	Amphipod <u>Pontoporeia hoyi</u>	DOC	4.8, 6.5, 7.4, ^a 11.24	no observed effect on k_u	Landrum et al. 1987
Benz[a]anthracene	Cladoceran <u>Daphnia magna</u>	DHM	0.3, 3.0, 30.0	calculated reduction in k_u = 6%, 38% and 86% respectively	McCarthy et al. 1985
Pyrene	Amphipod <u>Pontoporeia hoyi</u>	DOC	4.8, 6.5, 7.4, ^a 11.2	calculated reduction in k_u = 33%, 40%, 11% and 60% respectively	Landrum et al. 1987
Benzo[a]pyrene	Amphipod <u>Pontoporeia hoyi</u>	DOC	4.8, 6.5, 7.4, ^a 204	calculated reduction in k_u = 81%, 88%, 77.6%, & 81% respectively	Landrum et al. 1987
	Cladoceran <u>Daphnia magna</u>	DHM	0.15, 1.5, 15.0	calculated reduction in k_u = 17%, 68% and 95% respectively	McCarthy et al. 1985
	Bluegill Sunfish <u>Lepomis macrochirus</u>	DHM	1.0	calculated reduction in k_u = 67%	Spacie et al. 1983
	Bluegill Sunfish <u>Lepomis macrochirus</u>	DHM	20.0	calculated reduction in k_u = 90%	McCarthy & Jiminez 1985
3-methylcholanthrene	Cladoceran <u>Daphnia magna</u>	DHM	0.15, 1.5, 15.0	calculated reduction in k_u = 79%, 38% and 82% respectively	McCarthy et al. 1985

Footnotes: a - concentrations of DOC represent samples collected from different locations and during different seasons
DHM - Dissolved humic material
DOC - Dissolved organic carbon

McCarthy et al. (1985) described the influence of DHM on PAH uptake kinetics using a three compartment model (Figure 2.4.3). Partitioning of PAH between these compartments is governed by rate coefficients where K_u describes PAH uptake to the biotic compartment from the freely dissolved PAH pool, K_d represents depuration of PAH from the biotic compartment, K_s represents uptake of the complexed PAH and K_p describes sorption of freely dissolved PAH to DHM.

The net flux of PAH between these compartments is then described as:

$$dC_b/dt = (K_w C_w) + (K_s C_s) - (K_d C_b) \quad (2.4-1)$$

where C_b , C_w and C_s represent the concentration of PAH in the biotic, freely dissolved and sorbed phases, respectively.

Studies indicate that K_s for Daphnia magna exposed to naphthalene, anthracene, benz[a]anthracene, 3-methylcholanthrene and benzo[a]pyrene are essentially zero (McCarthy et al. 1985). Similar studies with bluegills (Lepomis macrochirus) also suggest K_s for naphthalene and benzo[a]pyrene is either very low or equal to zero, supporting the observation that upon complexation with DHM, PAH are effectively unavailable (McCarthy and Jimenez 1985).

The affinity of PAH to partition to DHM, or DOM in general, is correlated with the hydrophobicity of the compound (K_{ow} , octanol/water partition coefficient). A log-linear relationship exists between K_{ow} and K_p such that increasing hydrophobicity results in a greater degree of complexation with DHM. It follows, therefore, that the uptake of PAH (described by K_u) having low hydrophobicity (e.g. naphthalene vs benzo[a]pyrene) would be least affected by the presence of DHM, or DOC in general. This has been observed in several studies where K_u for naphthalene, anthracene and phenanthrene was unaffected by various concentrations of DHM (Table 2.4.6). Higher DHM concentrations (e.g. 30 to 60 mg C/L) eventually affected K_u for anthracene, however only minor DHM concentrations (e.g. 0.15 to 1.5 mg C/L) were required to evoke a substantial reduction in K_u for pyrene, 3-methylcholanthrene and benzo[a]pyrene by as much as 80%.

Because of the reduction in bioavailability, as measured by reduction in K_u , the bioconcentration factor (BCF) relative to total waterborne PAH also decreases. McCarthy et al. (1985) described a structure activity relationship to predict the modified bioconcentration factor (BCF_{MOD}) of PAH in aquatic organisms as governed by the partition.

coefficient for sorption (K_p) and concentration of DHM. Rearrangement of equation 2.4-1 yields the steady state BCF_{MOD} as follows:

$$BCF_{MOD} = \frac{[K_u + f(K_s - K_u)]}{K_d} \quad (2.4-2)$$

where f represents the fraction of total waterborne PAH bound to DHM, a function of K_p :

$$f = \frac{(K_p \cdot DHM)}{[1 + (K_p \cdot DHM)]} \quad (2.4-3)$$

Using the conservative assumption that K_s is approximately 5% of K_u , then

$$BCF_{MOD} = \frac{K_u}{K_d} \cdot (1 - 0.95f) \quad (2.4-4)$$

or

$$BCF_{MOD} = BCF_{NO\ DHM} (1 - 0.95f) \quad (2.4-5)$$

where $BCF_{NO\ DHM}$ is the BCF measured in the absence of DHM and is described by the empirically derived equation for Daphnia magna (McCarthy et al. 1985):

$$\log BCF_{NO\ DHM} = -0.20 + 0.654 \log k_{ow} \quad (2.4-6)$$

Substituting equations 2.4-3 and 2.4-6 into 2.4-5 yielded the function illustrated in Figure 2.4.4 (McCarthy et al. 1985) which exemplifies the sensitivity of compounds with large K_p (e.g. $\log K_p > 5.0$) while compounds having lower K_p (e.g. $\log K_p < 4.0$) are essentially unaffected by DHM.

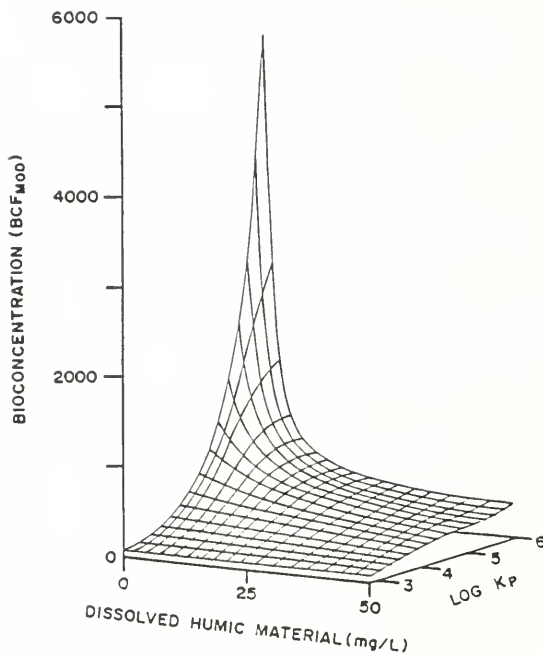


Fig. 2.4.4 Structure-activity relationship (SAR) between K_p , concentration of DHM, and BCF_{MOD} , based on Eqn. 2.4.5. The left edge of the figure describes the direct relationship between the hydrophobicity of the contaminant and its observed BCF in D. magna when no DHM is present. For a less hydrophobic PAH, such as naphthalene (front edge of the figure, $\log K_p = 3$), the BCF is low in the absence of DHM, and the presence of increasing amounts of DHM has relatively little effect. However, for a very hydrophobic compound like benzo[a]pyrene (back edge of the figure, $\log K_p = 6$), even small amounts of DHM bind a substantial fraction the contaminant, and this results in a large decrease in bioaccumulation.

(Modified from Landrum et al. 1987)

While the above relationship agreed well with the empirical data from which it was developed, it should be noted that it applies to the influence of DHM, as formulated through reconstitution of a standardized material (Aldrich Chemical Co.). Landrum et al. (1987) emphasize that such relationships, when extended to DOM in general, may not be accurate since the composition of DOM, and therefore complexing properties, may vary temporally and spatially. This was demonstrated by PAH uptake in the amphipod, Pontoporeia hoyi, using various sources of interstitial water as the exposure media (Table 2.4.6).

Landrum et al. (1987) also calculated a biologically determined partition coefficient for PAH binding to DOC (K_b , analogous to K_p described above). It was found, however, that K_b was not adequately predicted by K_{ow} , with only 46% of the variance in K_b explained (Figure 2.4.5). This inadequacy was partially attributed to the variation inherent in the sources of DOC used. The potential for error in prediction of K_b from K_{ow} was as high as two orders of magnitude, which infers poor predictability of the modified BCF. Thus, while K_{ow} was an adequate descriptor for K_p when associated with DHM of consistent composition, it clearly lacked this capacity for partitioning of PAH with variable DOC (K_b). Instead, K_b was more closely correlated with a sorption coefficient (K_{rp}) derived through reverse phase separation (Landrum et al. 1987). This descriptor explained 73% of the variation in K_b and represents a significant improvement in the ability to predict the modified BCF.

Complexation may also occur with sediments, and ingestion of PAH-bound particulates by benthic organisms such as amphipods and bottom feeding fish represents an additional source of exposure. Landrum and Scavia (1983) found that uptake of sediment-associated anthracene by the amphipod, Hyallella azteca, was considerably slower (K_u for sediment associated anthracene was 19 ± 5 g dry sediment $\cdot$ g animal wet wt⁻¹ $\cdot$ h⁻¹) than aqueous uptake (K_u for dissolved anthracene was 255 ± 76 ml $\cdot$ g animal wet wt⁻¹ $\cdot$ h⁻¹). However, sediment-associated anthracene was

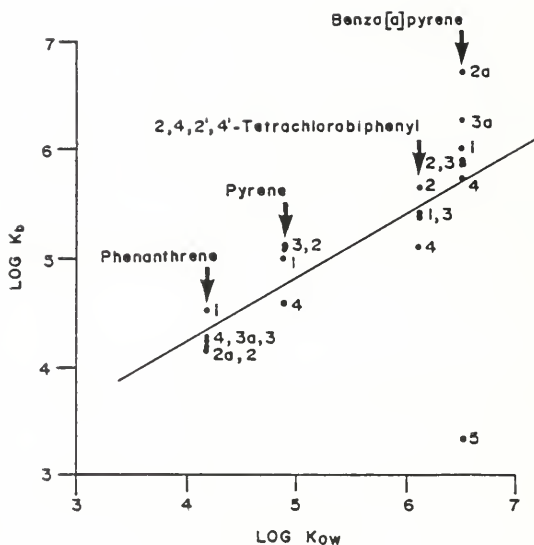


Fig. 2.4.5 Log-log scatter plot of the means of $\log K_b$ vs $\log K_{ow}$. The means are linearly correlated. However, the wide range of data for benzo[a]pyrene, $\log K_{ow}$ of 6.5 shows why $\log K_{ow}$ cannot be used to predict $\log K_b$. The number assigned to the data points represent different sampling locations. Sediments for interstitial waters from stations labelled 2a and 3a were collected in the spring along with Station 5. All others were collected in late summer and fall. ($\log K_b = 1.89 + 0.59 K_{ow}$, $r^2 = 0.46$, $n = 21$).

(Modified from Landrum et al. 1987)

estimated to account for 77% of the steady state body burden in these organisms. Therefore, although uptake from sediments is slower, it appears to be a more significant contribution to body burden, due primarily to the ingestion of contaminated particulates. The presence of organic sediment did not significantly influence aqueous uptake of anthracene, however, its presence increased depuration (K_d) by a factor of three. While natural sediments appear to evoke an increase in both total uptake and depuration of anthracene, the net effect is increased body burden, and therefore increased hazard to benthic versus free swimming organisms (Landrum and Scavia 1983).

The modifying effects of PAH complexation with DHM, DOC or organic sediments can be quite significant. By providing an "environmental sink" it may be regarded from one perspective that such partitioning mitigates the potential of the more hydrophobic PAH being transferred to higher trophic levels. On the other hand, this phenomenon should also stabilize the waterborne concentration of PAH by providing a long-term source from which PAH may desorb. This effect would then offset the attenuation of freely dissolved PAH through other removal pathways (e.g. bacterial decomposition and photo-oxidation). This process would therefore ensure long-term, low level exposure of aquatic biota to PAH.

2.4.1.4 Influence of Co-contaminants

Invariably contaminants other than PAH are also present in field situations, either in dissolved or sediment-associated form. In both cases, evidence suggests that certain co-contaminants may significantly modify the uptake, disposition, bioconcentration, metabolism and depuration of several PAH (Table 2.4.7).

Landrum (1983) investigated the role of low molecular weight co-contaminants on the bioavailability of PAH to the amphipod,

Pontoporeia hoyi. He determined that the uptake rate constant for a given compound (e.g. benzo[a]pyrene) was inversely proportional to the concentration of the co-contaminant (Figure 2.4.6). A unique concentration of co-contaminant could then be interpolated which resulted in 50% reduction in the uptake rate constant (I_{50}) which was reported for toluene, benzene, hexane, cyclohexane, ethylacetate and acetone (Table 2.4.7). For this same series of co-contaminants, in which aqueous solubility generally increases from toluene through to acetone, it was found that the ability to inhibit PAH uptake was compromised with increasing solubility of the co-contaminant, thus the trend towards increasing I_{50} 's in Table 2.4.7. Further studies indicated that for a given co-contaminant, the ability to inhibit uptake was reduced with increasing aqueous solubility of the primary contaminant (Table 2.4.8). These observations suggest that the ability of water to stabilize the primary contaminant in solution reduces the interaction of the co-contaminant with the PAH (Landrum 1983). Therefore, the more hydrophobic PAH (e.g. benzo[a]pyrene, $\log K_{ow} = 6.5$) would be most sensitive to this effect.

Several studies have effectively demonstrated that the presence of PCB's (e.g. Arochlor 1254) will influence the metabolite disposition and bioconcentration of relatively simple (2,6-dimethylnaphthalene) and complex (benzo[a]pyrene) PAH (Collier et al. 1985, 1986, Stein et al. 1984). Pre-exposure to Arochlor 1254 by i.p. injection significantly increased the metabolites of 2,6-dimethylnaphthalene in bile, but decreased its disposition in blood, brain and muscle of coho salmon (Collier et al. 1985). Similar effects were also noted for English sole exposed simultaneously to Arochlor 1254 and benzo[a]pyrene in spike sediments (Stein et al. 1984). Both studies proposed that MFO induction by the PCB resulted in a greater table 2.4.7

TABLE 2.4.7

INFLUENCE OF CO-CONTAMINANTS ON THE BIOAVAILABILITY AND METABOLISM OF PAHS TO AQUATIC BIOTA

CHEMICAL	CO-CONTAMINANT (exposure)	TEST ORGANISM	EFFECT	COMMENT ^d	REFERENCE
2,6-dimethyl- naphthalene	Arochlor 1254 (injection)	Coho Salmon <u>Oncorhynchus</u> <u>kisutch</u>	-PAH metabolite disposition	i.p. injection (100 mg/kg body wt) increased bile metabolites, but decreased metabolites in brain, blood and muscle	Collier <u>et al.</u> 1985
Anthracene	Toluene (waterborne)	Amphipod <u>Pontoporeia</u> <u>hovi</u>	-PAH uptake	$I_{50} = 2.2 \text{ mg/L}^b$	Landrum 1983.
	Oil Shale Retort Water (waterborne)	Rainbow Trout <u>Salmo gaird-</u> <u>neri</u>	-PAH uptake -PAH depuration -PAH bioconcen- tration	a concentration of 0.0045% of OSRW decreased K_1 by 25%, increased k_4 by 34% and de- creased the calculated steady state BCF by 40%	Linder <u>et al.</u> 1985
Benzo[a]pyrene	Toluene Benzene Hexane Cyclohexane Ethylacetate Acetone (waterborne)	Amphipod <u>Pontoporeia</u> <u>hovi</u>	-PAH uptake	-decrease in k_1 was inversely proportional to co-contaminant concentration - I_{50} for toluene, benzene, hexane, cyclohexane, ethylacetate and ace- tone was 0.74, 5.0, 2.5, 5.0, >10.0 and >100.0 mg/L, respectively	Landrum <u>et al.</u> 1983
	Arochlor 1254 (sediment)	English Sole <u>Parophrys</u> <u>vetulus</u>	-PAH metabolite disposition	-simultaneous exposure via sedi- ment increased tissue levels of B[a]P metabolites in skin, gill, liver and bile relative to B[a]P exposure alone	Collier <u>et al.</u> 1984
	Butadienes PCBs Pesticides (sediment)	English Sole <u>Parophrys</u> <u>vetulus</u>	-bioconcentration	-increased levels of B[a]P derived radioactivity observed in liver and bile after chronic exposure to organically contaminated sedi- ment relative to control sediment	Stein <u>et al.</u> 1986

TABLE 2.4.7 (cont'd)

INFLUENCE OF CO-CONTAMINANTS ON THE BIOAVAILABILITY AND METABOLISM OF PAHS TO AQUATIC BIOTA

CHEMICAL	CO-CONTAMINANT (exposure)	TEST ORGANISM	EFFECT	COMMENT ^a	REFERENCE
Benzo[a]pyrene (cont'd)	Arochlor 1254 (injection)	English Sole <u>Parophrys</u> <u>vetulus</u>	<u>in vitro</u> covalent binding	-liver supernatant of PCB-induced fish yielded higher covalent binding of B[a]P radioactivity to DNA	Egaas and Varanasi 1982
	Cadmium (injection)	Black Sea Bass <u>Centropristis</u> <u>striata</u>	MFO induction	-injection of 0.42 mg Cd/kg body wt decreased B[a]P hydroxylase activity by 50%, but no effect was noted for a dose of 2.5 mg Cd/kg	Fair 1986
	Cadmium (dietary)	Black Sea Bass <u>Centropristis</u> <u>striata</u>	-bioconcentration -MFO induction	-no effect of dietary cadmium (10 mg Cd/kg diet) on bioconcen- tration of B[a]P in selected tissues -no effect on B[a]P hydroxylase activity	Fair and Fortner 1987

Footnotes: a - All doses or exposure concentrations refer to the co-contaminant.

b - I_{50} refers to the concentration of co-contaminant which resulted in a 50% reduction in PAH uptake as measured by the uptake rate constant k_u .

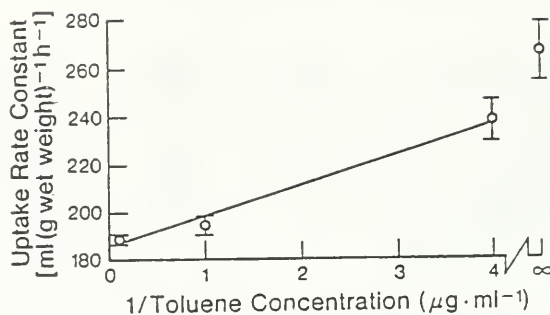


Fig. 2.4.6 Reduction in the uptake rate constant for benzo[a]pyrene by *Pontoporeia hoyi* with the inverse of the co-contaminant, toluene concentration. (Data represent mean $\pm$ 1SD).

(From Landrum 1983)

Table 2.4.8

CONCENTRATION OF TOLUENE RESULTING IN FIFTY PERCENT REDUCTION IN UPTAKE RATE CONSTANT OF PAH FOR PONTOPOREIA HOYI

COMPOUND	TOLUENE I_{50} * ($\text{mg} \cdot \text{L}^{-1}$)	WATER SOLUBILITY (21) ($\text{ng} \cdot \text{mL}^{-1}$)
Benzo[a]pyrene	0.74	3.6
Anthracene	2.2	74
Biphenyl	10 **	8080

* I_{50} = Concentration of co-contaminant resulting in a 50% reduction in uptake rate constant

** No effect at highest concentration tested $10 \text{ mg} \cdot \text{L}^{-1}$ of toluene

(From Landrum 1983)

production of benzo[a]pyrene metabolites. The high levels in the bile could then be explained by the hepatic clearance of the conjugated metabolite.

A similar influence was also noted for exposure of sediment-associated benzo[a]pyrene in the presence of sediment heavily contaminated with a myriad of organics (Table 2.4.7) (Collier et al. 1986). This study, however, involved a chronic exposure for 108 days, and resulted in significantly higher benzo[a]pyrene-derived radioactivity in both the liver and bile (Figure 2.4.7). These observations were again attributed to MFO induction by the co-contaminants, which then led to elevated production of metabolites and the potential for their retention through covalent binding.

It was also noted by Stein et al. (1984, 1986) that the tissue to sediment concentration ratios for PCBs significantly exceeded that for benzo[a]pyrene, however, only a small proportion of PCBs formed metabolites. In contrast, benzo[a]pyrene would be continually absorbed and metabolized to potentially carcinogenic and/or mutagenic compounds.

This theory is supported by the data of Egaas and Varanasi (1982). Their studies demonstrated pre-exposure of rainbow trout to Arochlor 1254, by i.p. injection, promoted in vitro covalent binding of benzo[a]pyrene radioactivity.

In contrast to the study of Stein et al. (1986), Linder et al. (1985) reported decreased uptake, increased depuration, and decreased bioconcentration of anthracene in rainbow trout exposed to the co-contaminant oil shale retort water. The difference may possibly be associated with the structure of anthracene, which lacks a bay region. Metabolites resulting from the induction of MFO would likely not have the propensity to form covalent bonds as would the more reactive diolepoxides of benzo[a]pyrene.

TABLE 2.4.9

INFLUENCE OF ROUTE OF EXPOSURE ON AQUATIC TOXICITY OF PAH COMPOUNDS

CHEMICAL	TEST ORGANISM	ROUTE OF EXPOSURE	EFFECT	COMMENTS	REFERENCE
Acridine	Fathead Minnow <u>Pimephales promelas</u>	i) Aqueous	Uptake	-BCF based on (i) and (ii) did not differ significantly, therefore contribution from sediment was negligible ($k_{\text{sediment}} = 0$) -uptake from (iii) insignificant	Southworth et al. 1979
		ii) Sediment/	Bioconcentration		
		Aqueous iii) Dietary			
Anthracene	Cladoceran <u>Daphnia magna</u>	i) Aqueous	Uptake	-the presence of sorptive yeast cells (ii) greatly decreased uptake rate and BCF -direct aqueous uptake is major route of exposure	McCarthy 1981
		ii) Dietary	Bioconcentration		
	Amphipod <u>Pontoporeia hoyi</u>	i) Aqueous	Uptake	-aqueous uptake ($k_u = 250 \text{ h}^{-1}$) was significantly higher than uptake from sediment and sediment ingestion (19.0 h^{-1}) -overall body burden was higher via (ii) -sediment associated anthracene accounted for 77% of BCF	Landrum & Scavia 1983
		ii) Sediment/ Dietary	Bioconcentration		
Naphthalene	Marine Copepod <u>Calanus helgolandicus</u>	i) Aqueous	Uptake	-aqueous uptake was poor in relation to dietary uptake -ratio of dissolved:food associated naphthalene concentration which yielded equivalent body burdens was 1743 to 3930	Harris et al. 1977
		ii) Dietary			
	Rainbow trout <u>Salmo gairdneri</u>	i) Aqueous	Metabolite disposition	-accumulation of naphthalene metabolites in skin accounted for (i) 52%, (ii) 17.6% and (iii) 15.1%, respectively	Varanasi et al. 1978
		ii) Dietary			
		iii) i.p. injection			
	Starry flounder <u>Platichthys stellatus</u>	i) Dietary	Biotransformation	-higher levels of naphthalene derived radioactivity retained in liver and bile of i.p. injected fish -greater degree of biotransformation resulted through (i) vs (ii)	Varanasi et al. 1979
		ii) i.p. injection	Metabolite disposition		

TABLE 2.4.9 (cont'd)

INFLUENCE OF ROUTE OF EXPOSURE ON AQUATIC TOXICITY OF PAH COMPOUNDS

CHEMICAL	TEST ORGANISM	ROUTE OF EXPOSURE	EFFECT	COMMENTS	REFERENCE
Benzo[a]pyrene	Cladoceran <u>Daphnia magna</u>	i) Aqueous ii) Dietary	Uptake Bioconcentration	-the presence of sorptive yeast cells (ii) greatly decreased uptake rate and BCF -direct aqueous uptake is major route of exposure	McCarthy 1981

Footnote: i.p. - intraperitoneal

Two other studies have addressed the influence of cadmium on metabolism and bioconcentration of benzo[a]pyrene in black sea bass (Fair 1986, Fair and Fortner 1987). Large i.p. injections of cadmium inhibited benzo[a]pyrene hydroxylase activity, however, pre-exposure via the diet (10 mg Cd/kg diet) had no effect (Table 2.4.7).

2.4.2 Biotic Modifying Factors

2.4.2.1 Age of Test Organisms

For many toxicants, age of the test organism is an important modifying factor of toxicity. Invertebrates and fish are commonly more sensitive to toxicants during the early stages of life, hence the use of early life stage tests (Sprague 1985, McKim 1985). Research addressing sensitivity of various life stages of aquatic organisms to PAH has not been extensive, however, several studies suggest the early life stages are more sensitive, and therefore pertinent from a regulatory perspective.

A review of the lethal toxicity of PAH to fish (section 2.1.1) revealed that embryo-larval exposures provided the most sensitive test organisms for acenaphthene, acridine, naphthalene and phenanthrene. Similarly, the data derived for invertebrates reveals first instar juveniles of Daphnia sp. were typically the most sensitive test organism. This observation, however, may also be a function of the sensitivity of the genus, in addition to age.

In addition to lethal toxicity, evidence suggests that juveniles are also more sensitive to sublethal chronic exposures. This is supported by the degree of covalent binding of benzo[a]pyrene metabolites to hepatic DNA of juvenile and adult English sole (Parophrys vetulus) as discussed in section 2.1.2.2.3. Juveniles demonstrated a covalent binding index at least two to four times that of adults, suggesting greater genotoxic sensitivity earlier in life (Varanasi et al. 1987).

The work of Metcalfe and Sonstegard (1984) and Black et al. (1985) illustrates the sensitivity and capacity for tumor induction within ten months in rainbow trout following a single embryonic injection of benzo[a]pyrene or dimethylbenzanthracene (section 2.1.2.3.1). Black's study generated a gross liver tumor incidence of 8.7% as a result of a 10 ug injection of B[a]P (136.1 mg/kg body wt). In contrast, Hendricks et al. (1985) observed an incidence of gross hepatic carcinomas of only 3% following a twelve month dietary exposure of rainbow trout fingerlings (3.0 g) to 1000 mg B[a]P/kg diet. If one assumes a daily ration of 2.5% body wet wt. over the duration of exposure, the exposure can be estimated to be approximately 25 mg B[a]P/kg body wet wt. While this is a lower dose than that implemented by Black et al. (1985), the duration of exposure is considerably longer, yet the frequency of gross hepatic carcinomas is approximately only one third that observed by Black et al. (1985). This may reflect a lower sensitivity to the carcinogen at the juvenile versus the embryo-larval stage.

Age dependent increases in tumor frequency have been observed in several fish populations affected with neoplasms (Black 1983, Varanasi et al. 1987). The higher frequency more likely reflects the time for development of the neoplasm as a result of earlier exposure, and is supported by the covalent binding index discussed earlier.

2.4.2.2 Route of Exposure

The manner in which PAH are introduced to an organism may influence bioavailability and metabolite disposition. Under field situations the routes of entry include direct aqueous uptake, uptake of sediment associated contaminant and dietary uptake. In addition, laboratory studies may involve intraperitoneal (i.p.) injection, where uptake then proceeds by transport across the gut wall and direct uptake into other organs. The influence of these routes of entry have been summarized in Table 2.4.9.

Results of studies conducted on invertebrates are variable. For the marine copepod, Calanus helgolandicus, direct uptake of dissolved naphthalene was poor in comparison to that achieved by feeding on contaminated diatoms (Harris et al. 1977). While the rates of uptake appeared comparable, the dietary route was far more important because it contributed to the total body burden to a greater extent.

Similarly, Landrum and Scavia (1983) found that ingestion of anthracene contaminated sediments had a greater influence on the calculated steady state BCF of the amphipod, Pontoporeia hoyi, even though the uptake rate constant from sediment ($K_s = 29.0 \text{ h}^{-1}$) was considerably less than that for direct aqueous uptake ($K_u = 255 \text{ h}^{-1}$). It would appear that in both of the above studies, direct aqueous exposure becomes limited by equilibrated uptake/depuration rates, a function of chemical and physical processes. However, dietary uptake is less governed by these processes, and influenced more by consumption of contaminated food, a process largely influenced by behavior. Thus, in Landrum's study dietary uptake of anthracene accounted for 77% of the calculated steady state BCF.

In contrast to the filter feeders above, direct aqueous uptake of anthracene or benzo[a]pyrene is reported to be far more important than dietary uptake in Daphnia magna (McCarthy 1981). This contradictory result may be a function of the biology of the cladoceran (e.g. lipid stores, surface to volume ratio) and/or the sorptive capacity of yeast food particles for the PAH.

Studies conducted on fish also demonstrate route of exposure influences PAH uptake, bioconcentration and metabolite disposition (Varanasi et al. 1978, 1979; Southworth et al. 1979b). In the case of acridine and naphthalene, direct aqueous uptake proved to be more relevant than sediment or dietary exposure for fathead minnows and rainbow trout (Table 2.4.8). Exposure of starry flounder to naphthalene demonstrated a greater degree of biotransformation via dietary uptake versus i.p. injection, however, higher levels of metabolites were observed in liver and bile of i.p. injected fish.

The difference in metabolite accumulation was thought to be due to biotransformation of naphthalene by intestinal bacteria during the dietary exposure.

Circumstantial evidence from various studies suggest i.p. injection to fish of PAH presents a lesser degree of acute challenge than aqueous exposure. This is exemplified by the tolerance of fish to high doses of various PAH for MFO induction studies (see section 2.1, Table 2.1.3).

2.4.2.3 Influence of Feeding

Food consumption during exposure to PAH can significantly modify the uptake and depuration kinetics of the xenobiotic. While this is identified as a separate modifying factor, it should be realized that this influence is closely associated with other factors such as complexation and route of uptake, already discussed.

Toxicokinetics of exposure to benzo[a]pyrene by bluegill sunfish (Lepomis macrochirus) was variably affected by feeding (Jimenez et al. 1987). In the absence of waterborne B[a]P, uptake from contaminated food over 48 h did not contribute significantly to the total ^{14}C B[a]P activity observed through aqueous uptake alone. However, when fish were fed and exposed to waterborne B[a]P, the uptake rate constant (K_u) was significantly increased from 15.4 (unfed) to 32.6 h^{-1} . Food consumption had an even greater influence on the rate of depuration, with a 10-fold increase in the depuration rate constant (unfed: $K_d = 0.0048\text{ h}^{-1}$, fed: $K_d = 0.054\text{ h}^{-1}$). This was also observed in fish fed only during the depuration phase (Figure 2.4.8).

The difference in kinetics between the fed and unfed regime is likely due to metabolite retention in bile, which is the primary storage site for metabolized and conjugated PAH. Because unfed fish are deprived

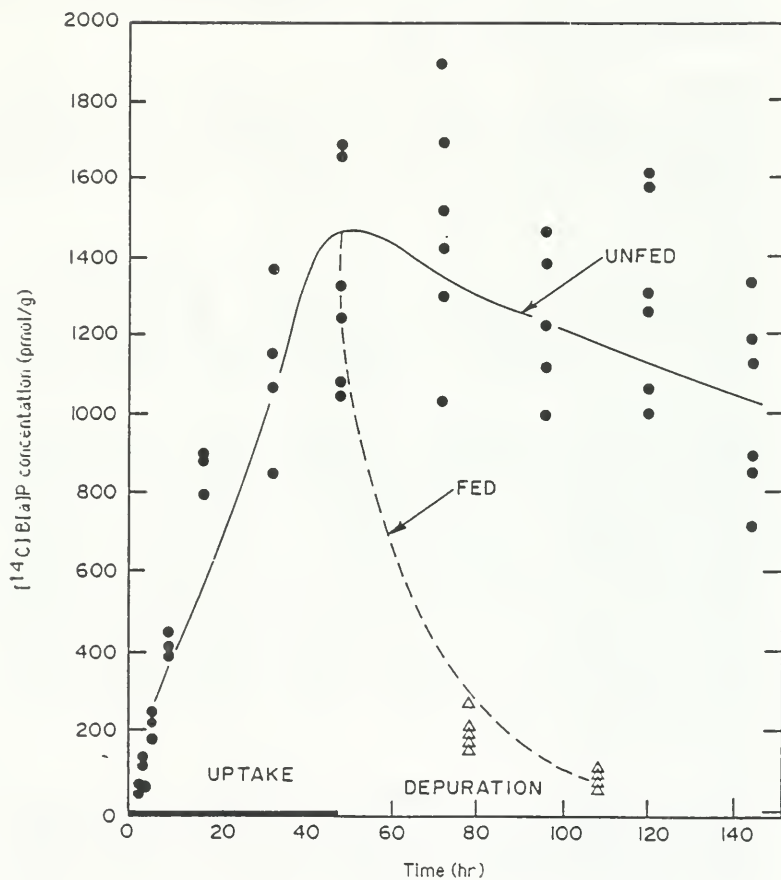


Fig 2.4.8 Uptake and elimination at 23°C of $[^{14}\text{C}]\text{B[a]P}$ bluegills fed during depuration (triangles) and unfed (circles) throughout the experiment. Uptake and depuration phases are indicated on the abscissa.

(From Jimenez et al. 1987)

of the digestive stimuli which release bile from the gall bladder, metabolite retention in the bile is prolonged. Fed fish, however, would receive these stimuli as food is digested, and hence, depuration of B[a]P and other conjugated PAH metabolites would be facilitated through the biliary-fecal route.

Since the depuration rate constant was increased to a greater extent than the uptake rate constant, it follows that steady state BCF would be reduced. Thus, the calculated steady state BCF for unfed fish was 3208, while that for fed fish was only 608.

Early life stages of organisms can be particularly sensitive to the influence of food availability on xenobiotic toxicity. The stress of toxicant challenge exerts an energy demand on metabolism, which may be mediated through a variety of pathways including increased respiration or detoxification and elimination (Pickering 1981). When endogenous energy stores are exhausted in larval fish or invertebrates, and exogenous food energy is unavailable or limited, early life stages may become vulnerable to this challenge. In the case of the calanoid copepod (Diaptomus clavipes), unfed nauplii exposed to acridine were significantly more sensitive than fed nauplii (Cooney and Gehrs 1984). Survival of unfed nauplii to the first copepodid stage, while exposed to 0.3 mg/L acridine, was only 15% of that for unexposed nauplii. Fed nauplii, however, could tolerate 1.0 mg/L acridine with no effects on survival at 16° or 21°C.

The influence of food consumption on toxicity and toxicokinetics of PAH can be significant as noted above. However, these studies do not address feeding behavior of exposed organisms, which is essential for an evaluation of field implications. No studies were found which addressed feeding behavior as modified by PAH.

2.5 ECOLOGICAL SIGNIFICANCE OF AQUATIC PAH TOXICITY

2.5.1 Photoinduced toxicity

An important concern associated with aquatic PAH contamination is the ecological significance of photoinduced toxicity. Numerous investigators have documented toxic impacts mediated through this mechanism at Solar Ultra Violet Radiation (SUVR) intensities equivalent to or less than the maximum which could be expected in field situations (Bowling et al. 1983, Landrum et al. 1986, Oris and Giesy 1987, Newsted and Giesy 1987). In addition, photoactivation renders certain PAH toxic well within the confines of their solubility, and at concentrations which may occur in field situations (e.g. interstitial waters of PAH-contaminated sediments).

The extent to which PAH photoinduced toxicity may proceed in surface waters will be governed by the incident SUVR intensity and its ability to penetrate to depths where organisms would be at risk. Intuitively, one would expect shallow clear streams to be most conducive to this phenomenon, and therefore all organisms noted in earlier sections could conceivably be at risk. In their discussion of this topic, Landrum et al. (1986) calculated that SUVR of sufficient intensity to cause 50% immobilization in the cladoceran, D. pulex, when exposed to anthracene (1.2 $\mu\text{g/L}$) over a 14 h daylight cycle, could penetrate to a depth of 7.0 m in Lake Michigan. This represents approximately one-fourth of the anthracene concentration which was photoactively lethal to fathead minnow larvae over 15.75 h (Oris and Giesy 1987). In addition, a depth of 7.0 m represents a sizable portion of the most productive habitat available to zooplankton and fish larvae.

Oris (1985) argued that on the basis of SUVR-induced stress alone, many aquatic organisms live at or near their UV tolerance limit. Any additional stress mediated through a similar mode of action (e.g. PAH photoinduced free radical generation via singlet oxygen) could very likely have adverse effects on these organisms.

Several other factors exist which warrant consideration. The first is the interactive phototoxic effects of numerous PAH compounds acting simultaneously on an organism. A single mode of action (free radical generation) suggests the effects would most likely follow an additive model. At this point, only a fraction of the known compounds have been investigated for photoinduced toxicity, yet it is plausible to expect many PAH to exhibit this phenomenon. As such, the potential for photoinduced toxicity in the field seems more likely.

An additional consideration is the potential for chronic sublethal effects experienced through this phenomenon. An important concept in this mode of action is that the majority of damage proceeds during SUVR exposure and not during the night, or, even in shaded areas subject to downstream flow of upstream photo-oxidized PAH (Oris 1985, Bowling et al. 1983).

Therefore the time spent in which PAH exposure is not concurrent with SUVR exposure can be regarded as "repair" time. The impacts become a function of accumulated tissue damage incurred over time versus the ameliorating effects of tissue repair. In the case of Ontario, it is likely that sublethal impacts (along with lethal impacts) would be most prominent during the long summer days when SUVR intensity and exposure time are maximized and repair time (i.e. dark cycle) is minimized.

It would be impractical to conduct large scale research on feral organisms to determine the extent of photoinduced toxicity in the field since the lesions are often nondescript. However, in view of the subacute responses evoked in spot (Leiostomus xanthurus) by highly contaminated sediments (see Section 2.1.2.2.3), it would seem warranted to pursue cage studies with organisms planted at sites known to be highly contaminated with PAH and at a depth conducive to SUVR penetration.

2.5.2 Toxicity to Fish

The data represents only a fraction of the hundreds of known compounds in this group, and are therefore an incomplete database from which to generalize. However, most other PAH are either rare enough in the environment that no threat to aquatic life has been perceived (and hence the lack of bioassays) or they are large and insoluble to the extent that negligibly low acute lethal toxicity may be assumed. For the PAH that have been tested, lethal concentrations are always orders of magnitude higher than concentrations found in even the most heavily polluted marine or fresh waters (Neff 1979, Eisler 1987).

Heavily polluted aquatic sediments, on the other hand, may contain PAH at concentrations equalling or exceeding levels acutely toxic to fish (Eisler 1987). The toxicity of PAH adsorbed to sediments is not thoroughly known; it is believed that these compounds are much less bioavailable than those in solution, and correspondingly less toxic, however, this is not always the case. As noted earlier, fish eggs and larvae are considerably more sensitive to PAH than adults. For eggs of largemouth bass, 7-day (3 days post-hatch) LC50's for acridine, naphthalene and phenanthrene were 0.91, 0.68 and 0.25 mg/L respectively, well below the LC50's reported for older fish (Table 2.1.1). For rainbow trout eggs, 27-day LC50's were even lower; 0.30, 0.12 and 0.03 mg/L respectively. These concentrations remain above the ambient concentrations found in polluted waters, but sediments in polluted regions may contain PAH concentrations in the range of 0.1 - 1.0 mg/kg (Eisler 1987). Since eggs of many fish incubate in stream-bottom gravel, there is a real possibility of exposure to PAH concentrations if the sediments are contaminated.

While the data on acute lethal toxicity to fish is limited, it has demonstrated a need for further research on compounds of interest. Additionally, it indicates a need for more studies to address the earlier life stages which are apparently most sensitive.

Sublethal effects of PAH to fish are of paramount importance, particularly due to the incidence of observed teratogenicity, mutagenicity and carcinogenicity. Teratogenic effects often result in death of newly hatched larvae, and since the frequencies for selected compounds may be high, this suggests that recruitment of larvae may be significantly compromised. This is possible, especially with eggs deposited in sediments of contaminated areas. Not only is direct uptake into the egg possible, as demonstrated by Hose et al. (1981), but observed embryonic MFO activity (Binder and Stegemen 1980, 1983) makes it highly likely that the accumulated PAH could be transformed to a reactive electrophile which is especially hazardous during embryonic developments. It should also be emphasized that in some locations maternal transfer of PAH may be an important initiating event of sublethal toxicity (Hose et al. 1981).

The carcinogenic hazard of many PAH has long been suspected to contribute to the observed frequency of tumours and neoplasia in feral fish. However, conclusive cause and effect relationships based on epidemiological studies will always be compounded by the variability in feral exposure due to the presence of other non-PAH carcinogens and the non-sessile nature of the test organism. Use of the ³²p postlabelling technique of DNA adducts suggests many of the DNA-adducts observed in feral fish exhibiting carcinomas are characteristic of PAH compounds (Maccubin et al. 1987). Although this data also does not unequivocally demonstrate a cause and effect relationship, it provides a measure of potential carcinogenic risk since DNA-adduct formation is regarded as a necessary initiating event in carcinogenesis. Since the majority of observed DNA-adducts are of a PAH nature this suggests that the long suspected carcinogenic hazard of environmental PAH is substantiated.

Chronic impacts of PAH on growth do not appear substantial and this end point is not a sensitive indicator of PAH impacts since subacute effects are also observed at concentrations influencing growth. Therefore, evaluation of ecosystem health with respect to

contamination would likely be more effective if approached on the basis of genotoxicity, carcinogenicity and reproductive failure rather than using age, weight and growth parameters.

2.5.3 Toxicity to Invertebrates

On the basis of conventional PAH toxicity, aquatic invertebrates appear, in general, to be more sensitive than fish. Evidence also suggests that the larvae and/or juveniles of some of these invertebrates (e.g. copepods and cladocerans) are more sensitive than adults (Cooney and Gehrs 1985, Cooney et al. 1984). While these observations suggest the potential for impacts at lower trophic levels, it is unlikely that field concentrations would be achieved which would evoke conventional lethal toxicity.

On the other hand, many of the PAH cited in Table 2.2.2 evoke photoinduced lethal toxicity at concentrations approaching 0.001 mg/L. Under these circumstances, environmental exposures could prove to be a significant problem.

Both modes of acute toxicity draw attention to the hazardous impact of the larger, more complex, compounds such as benz[a]anthracene and benzo[a]pyrene. These compounds have typically been of concern due to their inherent carcinogenic and mutagenic properties. The present data also suggest concern is warranted from the perspective of acute toxicity.

The information regarding sublethal acute and subacute effects is of limited value in making inferences regarding ecological implications since much of it is either based on marine studies or environmentally unrealistic concentrations. The chronic sublethal data clearly indicates that impacts on fecundity are the most relevant concerns, however, the data is limited to only a few compounds. Nevertheless, fecundity is an important factor in stabilizing lower trophic levels of the food chain.

The poor to lacking MFO activity in freshwater invertebrates is also an important factor since it enables very high bioconcentration factors to develop and therefore potentiate food chain transfer of unmetabolized PAH. This was well demonstrated in microcosm studies by Lu et al. (1977) where the BCF for B[a]P in Daphnia magna was approximately 134000 versus 930 for the mosquito fish (Gambusia affinis). A further implication of the low MFO activity in this group is that highly reactive electrophiles derived through oxidation and hydroxylation are less likely to occur and therefore the genotoxic and carcinogenic hazards may be considerably lower for this class of biota. However, such conjecture is based on a sparse database of MFO activity in freshwater invertebrates. In contrast, marine amphipods were observed to metabolize B[a]P when exposed to contaminated sediments and generated the same proximate carcinogens as fish and laboratory mammals. Additional research regarding MFO activity in these organisms is therefore warranted to determine if similar effects prevail in freshwater invertebrates.

2.5.4 Toxicity to Algae

Lethal toxicity of PAH to algae is poorly addressed in the literature and the available information describing lethal effects is based on environmentally unrealistic concentrations which approach the solubility limit of the PAH tested. Photoinduced toxicity of PAH to algae has been reported but contradictory studies suggest the effect on algae is not as significant as that observed in fish and invertebrates. Algal physiological processes (e.g. photosynthesis, nitrogen fixation) may be depressed by PAH exposure and this is important since any depression in primary production may have ramifications further up the food chain. However, some studies indicate the effects are reversible if the exposure is discontinued. While algae clearly are unable to avoid exposure as fish or invertebrates might do, the attenuation of dissolved PAH by such mechanisms as complexation and photo-oxidation following an episodic event may be a relevant factor in permitting primary production.

Several PAH were demonstrated to stimulate algal growth at low concentrations. While it has been postulated that this might reflect uncontrolled growth as in the case of neoplasia in animals, it may also be a hormetic effect in which algal metabolism over-compensates for a low level impact, with the result being enhanced growth. The implications of this effect may be to modify the population structure, but it is difficult to postulate to what extent this might happen and its ramifications on the food chain.

Perhaps the most important aspect of algal exposure to PAH is their ability to bioconcentrate PAH which may enhance food chain transfer. For example, Lu et al. (1977) reported a B[a]P bioconcentration factor in the algae Oedogonium cardiacum of 5258. This effect would potentiate the exposure of herbivores.

2.5.5 Modifying Factors

The most significant modifying factor which has environmental implications is that of photoactivation which has been addressed separately in Section 2.5.1.

The ability of PAH to complex with dissolved and particulate organic carbon has important implications. Firstly, it significantly reduces the bioavailability of PAH and may be important in some situations in ameliorating acute toxicity. However, a more serious implication is that the carbon pool can be regarded as a PAH sink from which PAH may desorb while remaining in equilibrium with the freely dissolved fraction. This effect results in stabilizing the dissolved PAH concentration and ensures a continuous low level exposure for biota.

The influence of co-contaminants is also an important factor since they may either ameliorate or potentiate toxicity. An especially important example of this is the enhanced uptake of PAH from sediment by English sole when PCBs are also present (Varanasi et al. 1987). In

addition to enhanced uptake, PCBs are efficient stimulators of MFO activity, and this dual effect promotes a greater degree of reactive metabolites being formed as observed in the covalent binding index for this fish (Section 2.1.2.2.2). The implications therefore suggest areas contaminated with both classes of contaminants may pose a greater genotoxic hazard to biota.

3. TERRESTRIAL TOXICOLOGY

The scope of this section was limited to wild and domestic animals and plants. It excludes all research on PAH which addresses experimental toxicology of conventional laboratory animals. The intent was to provide a database which would address environmental concerns associated with PAH exposure of the plants and animals indigenous to Ontario. It is clear, however, that large gaps in the database exist when laboratory animals are not considered. To some extent, this is not surprising since one of the purposes of experimental mammalian toxicology is to predict toxic impacts to other organisms.

It is clear however that wildlife toxicology of PAH requires a much needed effort to enable effective evaluation of the associated terrestrial hazards. The following sections address the available data with the structural confines of this report, although in many cases, information is completely lacking.

3.1 WILD AND DOMESTIC ANIMALS

3.1.1 Lethal Toxicity

No data was found regarding lethal toxicity of specific PAH to wild and domestic animals.

Related studies of lethal toxicity to farm animals includes accidental ingestion of kerosene or light sweet crude oil by calves. An ingested dose of light sweet crude oil equivalent to 40 ml/kg body wt. was lethal to calves over a three-day period (Rowe et al. 1973). A somewhat lower dose of kerosene, equivalent to 8 to 12 ml/kg body wt. per day, was also lethal to calves over a five-day period (Rowe et al. 1973). No information on the composition of the ingested dose was given, although kerosene contains much less PAH than crude or refined oils.

3.1.2 Sublethal Toxicity

3.1.2.1 Acute Effects

Information is restricted to that associated with the lethal toxicity above. Various signs associated with acute toxic impacts of kerosene or refined crude oil include anorexia, behavioral depression, decreased plasma glucose, loss of co-ordination, mental confusion and vomiting.

3.1.2.2 Subacute Effects

3.1.2.2.1 Genotoxic Effects

Field studies have recently employed similar measures of DNA adduct formation as already described for fish (Section 2.1.2.2.2). Talmage et al. (1986) measured the frequency of hemoglobin adducts formed with benzo[a]pyrene diol epoxides in several wildlife species (Table 3.1.1). The soil concentrations of B[a]P to which the animals were exposed ranged from 0.4 to 0.8 $\mu\text{g/g}$, while plants in the same area had a B[a]P content less than one-tenth of that in soil. The frequency of adducts appeared to be a function of food type and degree of soil contact, since the muskrat and shrew exhibited the highest frequency of adducts and also exhibit a high degree of soil contact (Talmage et al. 1986).

The demonstration of DNA/hemoglobin adducts in wildlife is a significant observation, since it indicates exposure, uptake and metabolism of B[a]P to a reactive metabolite has taken place. In addition, this measure of genotoxicity also enables comparison among organisms from different sites, and, in the case of hemoglobin adducts, repeated sampling of recaptured animals over a long period. Presently, however, the data is limited to only a few species, and additional work is required to determine the significance of various ranges of adduct frequency.

TABLE 3.1.1
CONCENTRATION OF BENZO[a]PYRENE ADDUCTS IN THE HEMOGLOBIN
OF SELECTED FERAL ANIMALS

Species	Number Captured	B[a]Adducts (ng/g Hb)
White-footed Mouse (<i>Peromyscus leucopus</i>)	15	0.00
House Mouse (<i>Mus musculus</i>)	1	0.00
Shorttail Shrew (<i>Blarina brevicauda</i>)	2	0.35
Norway Rat (<i>Rattus norvegicus</i>)	1	0.15
Muskrat (<i>Ondatra zibethica</i>)	6	0.23

(Data from Shugart et al. 1987)

3.1.2.2.2 Mixed Function Oxidase Activity

Reports of MFO activity in response to PAH contamination are lacking, although there is little doubt of its presence since it is well established in mammals and numerous other phyla. The previous discussion on B[a]P adduct detection in wildlife provides indirect proof of an active MFO system responding to an environmental concentration of a model PAH.

Studies conducted on domestic farm animals indicate an inducible MFO system in cattle (Shull et al. 1986, Jones et al. 1987). Both calves and adult cattle demonstrated a 3-methylcholanthrene-like induction of B[a]P hydroxylase, following subchronic administration of pentachlorophenol. The induction was present in both hepatic and pulmonary microsomes and was dose related.

Administration in food of tritiated 1,6[^3H]2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (75 $\mu\text{g/kg}$ body weight, p.o.) to lactating Holstein cows (500-650 kg) produced a dramatic increase in both aryl hydrocarbon hydroxylase and ethoxyresorufin-O-deethylation activity 7 days after treatment. A slight elevation in the activity of both enzymes was also observed 14 days after treatment with 0.05 μg TCDD/kg body weight (Jones et al. 1987).

Although there is only limited information, it is likely that PAH exposure will induce MFO activity. Further research is desirable to determine the extent of induction and also the availability of metabolites in milk from dairy cows as a function of MFO activity.

3.2 WILD AND DOMESTIC BIRDS

There are severe limitations in interpreting PAH toxicity data for wild bird populations since there is no knowledge of the organism's previous exposures to environmental contaminants. Previous exposure to PAH or other xenobiotics may have significant influence on subsequent PAH challenge. Nevertheless, results of the available studies have been addressed to provide some scope of understanding of the hazards associated with PAH and oil spills to birds.

3.2.1 Lethal Toxicity

The effect of oil mixture and PAH on eggs has principally been addressed by the application of the test solution applied externally or injected into the air cell end of the shell. Eggs are usually exposed at an age of 72 hours since embryotoxicity primarily occurs during the first three days of development (Hoffman, 1979, Hoffman and Campbell 1978a,b).

Egg mortality in mallard ducks (*Anas platyrhynchos*), was observed within 76 hours following application of #2 fuel oil. The mortality achieved was apparently dose dependent since a 5.0 μL application decreased the hatching success to 45% while a 50 μL dose resulted in complete mortality (Albers, 1977).

Embryotoxicity of a mixture similar to South Louisiana crude oil was shown to be dependent on the aromatic fraction (Hoffman and Gay 1981). Identification of individual oil components responsible for the mixture toxicity was attempted with a series of similar tests with Mallard eggs. 7,12-dimethylbenz[a]anthracene was more effective than benzo[a]pyrene or chrysene in enhancing the mixture toxicity. In all cases the progression of mortalities was the same as observed for crude oil, with a peak in mortality rate observed 72 hours after toxicant administration. However, a second period of mortality was observed on day 8-10, a few days after the growth of the choriallantoic membrane. It was suggested that enhanced uptake of the toxicants may have been mediated through this highly vascularized system (Hoffman and Gay, 1981). The same pattern of mortality was observed in Mallard eggs exposed to a complex mixture of PAH including dicyclic aromatics (20%), tricyclic aromatics (4%), tetracyclic aromatics (1%) and pyrene and aliphatic compounds. Addition of pyrene or chrysene to the mixture greatly enhanced embryotoxicity (Hoffman, 1979).

Additional attempts to identify the toxic components of Prudhoe Bay Crude and Fuel Oil #2 involved application of aromatic fractions to the air sac membranes of White Leghorn chicken eggs after removal of a small portion of the shell. The fraction containing di- and tricyclic compounds (naphthalene, biphenyl, fluorene, phenanthrene/anthracene, fluoranthene/ pyrene, and the 1H-Benzo[a]fluorene series) displayed greater embryotoxicity than tetra-and pentacyclic fractions (Ellenton 1982).

In another study, the LD50 of Prudhoe Bay crude oil and Hibernia crude oil applied to the shell of chicken eggs was found to be 1.3 and 2.2 μL respectively. The results suggest involvement of mixed function oxidase in the toxicity mechanism since disulfiram reduced embryo mortality from 60% to 20%. In rats, disulfiram reduces cytochrome P-450 levels suggesting that in the present study the impacts of reactive diol epoxides may have been ameliorated. However, further research is required to determine if the solvents utilized with disulfiram could also account for this observed reduction in crude oil toxicity (Lee et al. 1985). It is generally agreed that mortality does not occur as a result of a decreased rate of gaseous exchanges between the egg and the environment, since the amount of oil applied does not cover more than 5% of the egg shell surface. These studies indicate that oil mixtures as well as PAH can penetrate the egg barriers and that marked effects on embryo survival can be expected after a single exposure.

Very little information is available on the lethal toxicity of individual PAH towards juvenile or adult birds. Acute oral toxicity (LD50) of six PAH (9,10-dimethyl-1,2-benzanthracene, acenaphthene, phenanthrene, fluorene, indene and anthracene) was reported to be greater than 100 mg/kg body wt for all compounds using the redwinged blackbird, Agelaius phoeniceas, as the test organism (Schafer et al. 1983).

3.2.2 Sublethal Toxicity

3.2.2.1 Acute Effects

Bird embryos have proven to be a good model for the examination of acute embryo toxicity and teratogenic effects of PAH mixtures and several such studies have employed similar techniques (Hoffman 1979, Hoffman and Gay 1981, Ellenton 1982). The technique employed involves application of the toxicant in microliter quantities, either to the eggshell surface at the air sac end, or directly on the air sac

membrane following careful removal of less than 1 cm² of the shell. The time of application is critical in order to achieve influence during organogenesis. In the mallard duck (*Anas platyrhynchos*), this is typically at 72 h of incubation and 48 h for the chick embryo (Hoffman 1979).

The effects of a 10 µl application of an innocuous synthetic petroleum hydrocarbon spiked with either benzo[a]pyrene, chrysene or 7,12-dimethylbenzanthracene to mallard embryos are summarized in Table 3.2.1 (Hoffman and Gay 1981). These various measures of growth suggest embryonic exposure to PAH or petroleum fractions impose an energy burden resulting in less growth during this initial life stage. This may arise from energy expenditures mediated through MFO activity in detoxifying the xenobiotic which is well established in the avian embryo (Section 3.2.2.2.3).

TABLE 3.2.1
CONCENTRATIONS OF PAH ADDED TO SIMULATED CRUDE OIL WHICH EVOKED
SIGNIFICANT CHANGE IN THE NOTED RESPONSE VARIABLE^a

Response Variable ^b	% Composition of Synthetic Mixture		
	Benzo[a]pyrene	Chrysene	DMBA ^c
Embryonic Body Wt.	0.02 ^b	0.15	0.02 ^b
Crown-Rump Length	0.02 ^b	0.15	0.02 ^b
Bill Length	0.10	0.15	0.02 ^b

Footnotes : a - Eggs dosed with 10 µl hydrocarbon mixture on day 3 of incubation and assessed on day 18
b - Lowest dose tested
c - 7,12-dimethylbenz[a]anthracene

(Data from Hoffman and Gay 1981)

3.2.2.2 Subacute Effects

3.2.2.2.1 Teratogenic Effects

As an extension of the previous study, embryos were assessed for abnormal features which included stunting, eye, brain and bill defects and incomplete ossification as a result of the three PAH spiked at levels representative of some crude oils. Using the same technique, Hoffman and Gay (1981) reported similar effects in mallard embryos resulting from application of microliter quantities of crude and refined oils. He also determined that the teratogenicity could be attributed to the polyaromatic fraction and not the aliphatic fraction.

Ellenton (1982) also employed fractionation of Prudhoe Bay Crude and fuel oil No. 2 for applications to the chick embryo. The greatest teratogenicity was associated with the fraction containing 2-3 ringed aromatics which included alkylated naphthalenes, anthracenes, phenanthrenes, fluorenes and biphenyls. The frequency of abnormal embryos resulting from this treatment reached a maximum of 71% for a treatment of 2.5 mg-equivalents. The fraction containing 4-5 ringed aromatics included alkylated chrysenes, benz[a]anthracenes, triphenylene, phenylanthracene, benzo[a]pyrenes, perylenes and benzo[a]perylene. However, this fraction did not exhibit significant increases in teratogenic effects even at the maximum application rate of 5 mg-equivalents. The most commonly observed anomalies observed in this study included gastroschisis, edema, beak abnormalities, stunted growth, retarded feathering, hemorrhages, limb defects, hydrocephaly, craniocoles, retarded ossification, anophthalmia and fusion of the vertebrae (Ellenton 1982).

These studies demonstrate the possibility for crude oil fractions and isolated PAH to evoke teratogenic responses from avian systems when subjected to minute external applications. The system is attractive, however, it presently lacks solid dose-response data which is based on isolated contaminants and defined doses. As such the data can only be regarded as secondary in quality for regulatory purposes.

3.2.2.2.2 Genotoxic Effects

Bloom (1984) described extensive work conducted on the action of genotoxic agents toward chick embryos using essentially the same exposure technique described for studies of teratogenicity. The xenobiotic is administered at 74 h incubation time directly onto the inner shell membrane. Two hours prior to sacrifice (94 h) colcemid is applied to arrest cell mitosis in metaphase for enumeration of chromosomal aberrations, or 5-bromodeoxyuridine is injected for the detection of sister chromatid exchanges. Table 3.2.2 summarizes the lowest observable effects concentration (LOEC) for a variety of mutagenic PAH. The LOEC in this case, however, does not follow the criteria for aquatic toxicity as described by Stephan et al. (1985) which addresses growth, reproduction and survival. Nevertheless, the present data is viewed as a reliable measurement of a hazardous impact to a model avian embryo and is useful from a regulatory perspective.

TABLE 3.2.2
LOWEST OBSERVABLE EFFECTS CONCENTRATION (LOEC) FOR SIGNIFICANT
INDUCTION OF SISTER CHROMATID EXCHANGES IN CHICK EMBRYOS

Chemical	LOEC ($\mu\text{g}/\text{embryo}$)
2-aminoanthracene	1.0
benzo[a]pyrene	1.8
2-acetyl amino fluorene	2.5
9,10-dimethyl-benz[a]anthracene	4.0

(Data from Bloom 1984)

Additional genotoxic data based on SCEs was reported for a series of promutagenic PAH. Because of the exceptionally well developed MFO system in avian embryos (see Section 3.2.2.2.4) activation of promutagens to mutagens proceeds effectively in this test model.

Table 3.2.3 summarizes the LOECs for promutagenic PAH in avian embryos. These data are again regarded as reliable measurements of the associated genotoxic hazards associated with these compounds in the present avian embryonic model.

3.2.2.2.2 Mixed Function Oxidase Activity

Recently a number of studies have reported that the microsomal MFO capacity of the chick embryo is well developed (Hamilton et al. 1983, Hamilton and Bloom 1986, Gamett and Klein 1983, Lee et al. 1986, Bloom 1984) and it is believed that this activity plus the sensitivity of embryonic development may largely account for the high sensitivity of various avian embryos to petroleum hydrocarbon toxicity (Lee et al. 1986).

TABLE 3.2.3
LOWEST OBSERVABLE EFFECTS CONCENTRATION (LOEC) FOR SIGNIFICANT
INDUCTION OF SISTER CHROMATID EXCHANGES IN CHICK EMBRYO
AFTER MFO INDUCTION

Chemical	MFO Inducer Employed ^a	LOEC (μ g/embryo)
2-aminoanthracene	PB	0.38
benzo[a]pyrene	AR	0.71
2-acetylaminofluorene	AR	0.98
9,10-dimethyl-benz[a]anthracene	AR	1.64

Footnote: a - MFO inducing agent: Phenobarbital (PB)-for P-450
metabolism Arochlor (AR)-for P-448 metabolism

(Estimated from Bloom, 1984)

Lee et al. (1986) characterized the enzymes in the chick embryo for their inductibility at 5 days incubation time. The enzymes were maximally induced using Prudhoe Bay Crude oil after 24 h and included cytochrome P-450 (fourfold), naphthalene hydroxylase (sixfold), benzo[a]pyrene 3-hydroxylase (14-fold) and 7-ethoxyresorufin-O-deethylase (24-fold). Hamilton et al. (1983) noted that AHH activity was induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin, 3-methylcholanthrene, beta-naphtha-flavone and phenobarbital. In addition, activity was detected in undifferentiated embryonic tissue as early as three days incubation. This activity was equivalent to activity found in adults, and was present in tissues prior to the differentiation of liver which typically occurs by day five of incubation. The capacity for MFO induction above basal activity was apparent only after the liver had differentiated, and reached a maximum 30-fold increase between days 7 and 10 of incubation.

Because the chick embryo has an active, uninduced ability to metabolize PAH, this results in generation of reactive intermediates which are embryotoxic and carcinogenic (Hamilton et al. 1983). It was found that DNA damage in the liver correlated with induction studies, however, DNA damage in the lung, another site having MFO activity, did not correlate with induction studies (Hamilton et al. 1986).

Presently, further characterization of this embryonic activity is required and this should include characterization of all metabolites. In addition, the aspect of differential tissue sensitivity should also be addressed in order to better understand the overall sensitivity of avian embryos to petroleum hydrocarbons and PAH.

3.2.2.3 Chronic Effects

3.2.2.3.1 Growth and Reproductive Effects

Growth

In addition to impaired growth as an acute response to embryotoxic exposures, chronic exposures also affect growth. Feeding of various volumes of South Louisiana Crude oil weathered over sea water for 36 h to black guillemots (Ipphus grylle) produced a significant (20%) decrease in weight gain rate over the 22 days of experimentation (Peakall et al. 1980). Prudhoe Bay Crude oil administration (single dose of 0.2-2 g/kg body weight) reduced weight gain of nestling herring gulls (Larus argentatus) and induced a transient impairment of their osmoregulatory capacity (Peakall et al. 1982). They identified the high molecular weight fraction of Prudhoe Bay Crude oil as being responsible for these effects.

In a long-term study, wild mallard ducks (A. platyrhynchos) were fed food contaminated with a complex mixture of PAH over a 5 month period. An initial decrease in growth rate was observed for the first two months of exposure, however, growth rate recovered to a normal level by the end of the experimental period. Although food consumption was not measured, it was believed the toxicant effect was mediated through a decrease in the voluntary intake by the birds because of reduced food palatability (Patton and Dieter 1980).

The observed decrease in growth rate of birds suggests an interaction with energy metabolism. Although the consequences of these observations appear rather limited over the confines of the study period, they may become significant over a longer period of time. For example, depressed energy availability or storage might impair the ability of birds to perform in stressful circumstances such as migration or overwintering.

Reproduction

Reproduction in birds can be seriously affected by oil exposure. Feeding of South Louisiana Crude oil contaminated food to mallard ducks increased the time to gonadal maturation. The hatchability of eggs incubated by contaminated ducks was also reduced. It was suggested that crude oil effects on reproduction are mediated through an impairment of normal hormonal cycles (Cavanaugh et al. 1983). However, the use of crude oil makes it impossible to attribute the effects to PAH per se.

These results stress the need for further research in the light of the potential threat to water fowl. Further research is also needed regarding the impacts of PAH on the reproduction of other bird species.

3.3 REPTILES AND AMPHIBIANS

3.3.1 Lethal Toxicity

There is limited information on the lethal toxicity of PAH to amphibians. Most investigations are limited to complex mixtures of PAH and other compounds (Dumont et Schultz 1980, Schultz et al. 1982, Schultz et al. 1983).

Lethal toxicity of acridine was evaluated in embryos of Xenopus laevis at different developmental stages. The 96-h LC50 was 4.5 mg/L and all embryonic stages were affected identically.

The lowest concentration causing 100% mortality after 120 h of exposure in mid blastulae embryo was 5.0 mg/L acridine. Although there was only 8% mortality in the first inferior treatment level (3.0 mg/L acridine), the high proportion of abnormal survivors (75/92) suggests a failure of the recruitment process since abnormal larvae might die before reproducing.

3.3.2 Sublethal Toxicity

3.3.2.1 Subacute Effects

3.3.2.1.1 Teratogenic Effects

Numerous teratogenic effects of PAH have been observed. Damage in Xenopus embryos exposed to different concentrations of coal-gasification sour water, ranged from minor tail flexure to gross distortion of the longitudinal axis as well as optic, cephalic, visceral, and pericardial tissues (Dumont and Schultz 1980). Although these authors worked with a complex mixture of chemicals, they suggested that an association between the teratogenic effect and the PAH fraction existed.

Exposure of the embryos of amphibian Xenopus (mid-blastulae) to acridine concentrations as low as 1 mg/L, displayed pathological damage of the spinal cord and musculature. This induced damage, immobilized the larvae and suggests that teratogenic effects can occur at low concentrations (Davis et al. 1981).

3.3.2.1.2 Genotoxic Effects

The current understanding of PAH-induced DNA damages in amphibians involves two responses: a "fast effect" and a "delayed effect". DNA damage, measured as single-strand cleavable sites using electron microscopic evaluation, was observed in toads (Xenopus laevis) within 30 to 90 minutes of injection with 1 µg benzo[a]pyrene/g of body weight and disappeared within three hours of dosage ("fast response"). This effect could not be attributed to MFO metabolites since the enzymatic activity was not detected. However, depending on the dosage, occurrence of more extensive damage was observed several days later with a concurrent increase in MFO activity (Hohn-Bentz et al. 1983, Zahn et al. 1984, Ruben and Balls 1964).

These observations however are of limited usefulness in evaluating the carcinogenicity of PAH in amphibians since neither the dose nor the mode of administration is representative of environmental conditions.

3.3.2.1.3 Mixed Function Oxidase Activity

The ability of leopard frog (Rana pipiens) and garter snake (Thamnophis) liver to metabolize benzo[a]pyrene is less than that of rat liver. Frog cytochrome P-450 content is greater than that found in the snake, yet the specific activity of the benzo[a]pyrene hydroxylase in frog liver is approximately equivalent to that found in the rat (Schwen and Mannering 1982). Administration of 3-methylcholanthrene increased the benzo[a]hydroxylase activity in both the frog and snake. The specific activity of the enzyme was not modified, however, different species of cytochrome P-450 were produced in both test organisms. This induction persisted several weeks after the animals received a single dose of 3-methylphenanthrene (40 mg/kg) (Schwen and Mannering 1982).

The specific activity and content of both cytochrome P-450 and naphthalene hydroxylase in toad (Xenopus laevis) liver is approximately 30-40% of that found in male rats or mice, and 54% of that found in the bluegill Lepomis macrochirus. The levels are low in larvae but begin to increase after metamorphosis occurs and the adult level is reached (Doherty and Khan (1981).

The relatively low content of monooxygenase system may predispose these lower vertebrates to greater risk due to bioconcentration of the toxicants and a poor ability to metabolize and depurate the xenobiotics. However, since PAH carcinogenicity is attributed to the metabolites produced upon exposure (Jerina and Daly 1974, Jerina et al. 1976, Huberman et al. 1976), the low enzymatic activity still warrants the investigation of the potential carcinogenicity through generation of reactive intermediates.

There are numerous reports of experimental tumor induction by either injection or implantation of PAH crystals (among others; de Lustig and Matos 1970, Matos and de Lustig 1973, Balls 1963, Seilern-Aspang and Dratochwil 1962), however, these studies have no environmental application. Rate of regeneration from an amputated limb is either reduced or enhanced by PAH administration (Pizzarello and Wolsky 1966).

3.4 TERRESTRIAL INVERTEBRATES

3.4.1 Lethal Toxicity

Polynuclear aromatic hydrocarbons are frequently transported and are subject to occasional spills which enters the environment and could jeopardize organisms in the spill area.

Contact toxicity tests were conducted using filter paper soaked in toxicant solution to evaluate the lethal toxicity of different PAH to the worm Eisenia fetida, which are important in maintaining productivity and fertility of the soil. Should an accidental spill occur over a wide area, the susceptibility of worms to PAH toxicity, could reduce the rate of recovery of the terrestrial ecosystem from the spill. Some of the PAH were classified as being very toxic (acenaphthene, 48-h LC50 $49 \mu\text{g}/\text{cm}^2$), while others were found to be relatively nontoxic (naphthalene, 48-h LC50 $4670 \mu\text{g}/\text{cm}^2$) (Neuhauser et al. 1985). In another series of contact tests, benzo[a]pyrene was observed to be relatively nontoxic (48-h LC50 $> 1,000 \mu\text{g}/\text{cm}^2$) (Roberts and Dorough, 1984). The results of these toxicity tests suggest that unlike aquatic toxicity, terrestrial invertebrate toxicity cannot be correlated with either the log K_{ow} value, the vapor pressure, nor with the log of water solubility (Neuhauser et al. 1985).

Another test procedure that was used to assess the effect of exposure by the worm to a toxicant was to develop an artificial soil to which the toxicant could be added. This approach more closely resembles the conditions which prevail in the environment. Fluorene was the only PAH tested in this manner and was found to have a 48-h LC50 of 173 mg/kg of contaminated soil (Neuhauser et al. 1985).

First instar larvae of Drosophila melanogaster (strain zeste) were exposed to concentrations of 0.1 to 1.0 mM acridine orange in the growth media with which they had direct contact. Treatments produced a concentration-dependent reduction in the viability of the larvae. Mortality ranged from 42 to 98% (Xamena et al. 1984).

Contact tests conducted on cricket eggs (Acheta domesticus) with soil samples contaminated by a synthetic fuel spill indicated that the fuel was extremely toxic to this test organism. Furthermore, soil from the spill site (collected 6 months after the event) was more toxic than sand dosed with the synfuel mix (Strayer et al. 1983). Unfortunately the researchers did not analyze the toxicant composition of the sand mix, and therefore all of the toxicity cannot be conclusively attributed to PAH toxicity.

3.4.2 Sublethal Toxicity

3.4.2.1 Subacute Effects

3.4.2.1.1 Teratogenic Effects

Soil samples contaminated with high concentrations of a Synfuel oil blend (synthetic fuels), collected 6 months after a spill, were still teratogenic to crickets eggs (Acheta domesticus). Prepared experimental soil samples containing a 0.05% proportion of the contaminated soil sample, induced a significant increase in the

frequency of extra eye spot. These results however, should be viewed with caution since they pertain to a mixture of compounds which were not analyzed (Strayer et al. 1983).

3.4.2.1.2 Genotoxic Effects

Most sublethal PAH toxicity studies with terrestrial invertebrates has been conducted with the aim of evaluating the mutagenic and teratogenic potential of the compounds tested, in order to identify potential carcinogens.

Preliminary results indicated that both 2-acetylaminofluorene and benzo[a]pyrene have limited potential as precarcinogenic agents in adult Drosophila since a sex-linked recessive lethal assay elicited no response. However, introduction of either compound into the growth medium at an unspecified concentration produced an increase in sex-linked recessive lethality to third instar larvae (Angus 1984). This suggests early life stages of terrestrial invertebrate are more sensitive as already observed for aquatic invertebrates and fish.

3.4.2.1.3 Mixed Function Oxidase Activity

Biotransformation performed by mixed function oxidase (MFO) is primarily performed by the cytochrome P-450 enzyme system. The level of the different enzymes is determined both genetically and by the pre-exposure of an organism to inducing substances.

There was no information on the MFO induction by PAH exposure in terrestrial invertebrates in the literature, however, pre-exposure to other xenobiotics, such as phenobarbital or Arochlor 1254, was reported to stimulate benzo[a]pyrene monooxygenase activity in Drosophila (Hallstrom et al. 1984). Marked

differences in benzo[a]pyrene monooxygenase activity were observed between strains of exposed flies. The Hikone R strain had a capacity to metabolize benzo[a]pyrene at concentrations six times less than the other tested strains.

3.5 NATURAL VEGETATION AND FIELD CROPS

3.5.1 Lethal Toxicity

The lethality of PAH to higher plants is not well addressed in the literature: there are no reports of a lethal PAH dose in the reviewed literature. The Ontario Ministry of the Environment report on backyard garden damage from a nearby roofing operation (Emerson 1981) specifies that the exposure was lethal to some tissues (leaves and petals), but that the plants recovered. This is most likely due to the regenerative abilities of many higher plants. Apical meristems, crowns, axillary buds, stolons and tubers often remain viable despite deterioration of the plant due to various stresses.

3.5.2 Sublethal Toxicity

3.5.2.1 Subacute Effects on Plant Growth and Physiological Processes

Data describing growth effects of PAH on higher plants are scant, and much of it is of limited use, as the studies used excised sections of plants or germinated seeds (Table 3.5.1). It is very difficult to extrapolate data from the response of these tissues to whole plant response. The effect of exposure to PAH prior to germination of seeds has been investigated in several species. Benzo[a]pyrene, at a low concentration (0.5 $\mu\text{g/L}$) appeared to stimulate corn root growth (shoot growth was unchanged) following the soaking of seed for 6 or 12 h; wheat shoot growth was unaffected (Deubert et al. 1979). Fujii and

Table 1.5.1: Subacute Effects on Plant Growth and Physiological Processes

Chemical	Test Organism	Life Stage or Size	Method	Response Variable	Effect	Exposure Concentration (mg/L)	Exposure Duration	Reference
Acenaphthene	Maize	Young root tips	GL ^a	Root tip elongation rate	slowed	15.4	5 hours to 7 days	Shirk and Appleman, 1940
				Respiration rate	slowed	15.4	5 hours to 7 days	Shirk and Appleman, 1940
Anthracene	Maize	Young root tips	GL	Root tip elongation rate	no effect	17.8	5 hours to 7 days	Shirk and Appleman, 1940
				Respiration rate	no effect	17.8	5 hours to 7 days	Shirk and Appleman, 1940
Benzo[ghi]perylene	Zucchini	Mature plants	FC ^a	Tissue concentration to injured foliage	122.0 vs. 10.95 ng/g ^c (injured vs. healthy)	unknown	unknown (probably 1 or 2 days)	Emerson, 1981
				Shoot growth	no effect	.0005 to .02	6 to 12 hours	Deubert et al., 1979
Benzol[a]pyrene	Corn	Seeds	CL	Shoot growth	stimulated at lowest concentration	.0005 to .02	6 to 12 hours	Deubert et al., 1979
				Mutagenic spotting of foliage	no effect	5 to 100	24 hours	Fujii and Inoue, 1985
Benzol[k]pyrene	Zucchini	Mature plants	FC	Shoot growth	no effect	.0005 to .02	6 to 12 hours	Deubert et al., 1979
				Tissue concentration to injured foliage	305.5 vs. 2.79 ng/g (injured vs. healthy)	unknown	unknown (probably 1 or 2 days)	Emerson, 1981
Benzol[k]pyrene	Zucchini	Mature plants	FC	Shoot growth	no effect	.0005 to .02	6 to 12 hours	Deubert et al., 1979
				Tissue concentration to injured foliage	596.5 vs. 7.83 ng/g (injured vs. healthy)	unknown	unknown (probably 1 or 2 days)	Emerson, 1981

Table 3.5.1: Subacute Effects on Plant Growth and Physiological Processes (cont'd)

Chemical	Test Organism	Life Stage or Size	Method	Response Variable	Effect	Exposure Concentration ($\mu\text{g/L}$)	Exposure Duration	Reference
Dibenz(a,b)anthracene	Corn	Young root tips	RL	Root tip elongation rate	no effect	22.8	5 hours to 7 days	Shirk and Appleman, 1970
				Respiration rate	no effect	22.8	5 hours to 7 days	Shirk and Appleman, 1970
Fluoranthene	Zucchini	Mature plants	FG	Tissue concentration in injured foliage	1618 vs. 9.36 ng/g (injured vs. healthy)	unknown	unknown (probably 1 or 2 days)	harrison, 1981
Methylcholanthrene	Corn	Young root tips	RL	Root tip elongation rate	reduced ^c	25.5	5 hours to 7 days	Shirk and Appleman, 1970
				respiration rate	reduced	25.5	5 hours to 7 days	Shirk and Appleman, 1970
Pyrene	Zucchini	Mature plants	FG	Tissue concentration in injured foliage	691.5 vs. 8.35 ng/g (injured vs. healthy)	unknown	unknown (probably 1 or 2 days)	Everson, 1981
Phenanthrene	Zucchini	Mature plants	FG	Tissue concentration in injured foliage	202.4 vs. 6.42 ng/g (injured vs. healthy)	unknown	unknown (probably 1 or 2 days)	Everson, 1981

Footnotes: a - RL = Root exposure in liquid culture

b - FG = Foliage exposure to gas

c - G = Fresh weight

Inoue (1985) observed that soaking seed in B[a]P for 24 h at 5 - 400 mg/L did not result in mutagenic spotting of soybean seed. These two studies demonstrate that exposure of seeds to B[a]P has little effect on plant growth.

The exposure of excised maize root tips to several PAH (5 h - 7 d) demonstrated that anthracene (17.8 mg/L) and dibenz[a,h]anthracene (22.8 mg/L) had no effect on elongation or respiration, while acenaphthene (15.4 mg/L) and methylcholanthrene (26.8 mg/L) reduced both parameters (Shirk and Appleman, 1940). The final study in this section is an OME caselog of vegetable garden injury following an accidental industrial release (Emerson, 1981). A broad spectrum of vegetable species was injured by exposure to at least six PAH; these plant species demonstrated accumulation of the compounds (see Table 3.5.2). Although there is no air quality data with this study, the tissue PAH concentrations of healthy and injured foliage was contrasted for zucchini only. Fluoranthene (1.614 $\mu\text{g/g}$ fresh weight) was the most abundant PAH in the injured tissue, followed by perylene, benzo[k]pyrene, benzo[a]pyrene and o-phenanthrene. The most prevalent compound in healthy tissue was the least prevalent in injured tissue (benzo[ghi]perylene: 24% (0.122 $\mu\text{g/g}$) vs 3% (0.011 $\mu\text{g/g}$) of total PAH). It is not possible to infer from this data whether a single PAH or a PAH profile was responsible for the observed phytotoxicity. There were no primary studies in this section, as either excised sections of plants were used, or the exposure dose was not known.

3.5.2.2 Higher Plant Toxicokinetics

3.5.2.2.1 Bioaccumulation of PAH in Higher Plants

The studies describing the bioaccumulation of PAH in higher plants include surveys of garden vegetables, dietary components, and semi-controlled experiments using soils supplemented with soil conditioners containing PAH (Table 3.5.2). The ambient surveys are of little use,

except to demonstrate the wide range in concentrations, depending on the chemical and plant species (Pyysalo 1979, Dennis et al. 1983, Kveseth et al. 1981). Kveseth et al. (1981) suggest that low molecular weight PAH would tend to be absorbed by plant tissue, whereas those PAH of higher molecular weight, which are associated in the atmosphere with particulate matter, would be surface contaminants, available for removal by rain or washing. Edwards (1983) reported that PAH uptake rates by plants was dependent on the nature of the plant growth substrate, PAH solubility, PAH phase (vapor or particulate) and molecular weight.

The surveys of plants grown on PAH enriched soil demonstrate some interesting trends. Potato tubers accumulate B[a]P, B[e]P, and B[b]k]F to approximately one tenth of the concentration which was detected in haulms (underground stems). This was in contrast to perylene, indeno[1,2,3-cd]pyrene, D[ah]A and B[ghi]perylene which demonstrate haulm:tuber concentration ratios ranging from 2 to <1.

Some of these studies may be used to calculate soil:plant concentration ratios, but their interpretation should be cautious. Most of the studies also allowed the exposure of plants to ambient air, and fail to describe whether samples were washed to remove surface adhered PAH prior to tissue analyses. In general, the ratios were very high, which is consistent with the work of Edwards et al. (1982; section 3.5.2.2) who observed that anthracene in soil is quite unavailable for root absorption, in comparison to its availability in hydroponic solution. The tendency of a soil to bind PAH would probably relate to its content of organic matter and cation exchange capacity. Soil:plant concentration ratios ranged from <2 to 1,500 (Muller 1976, Linne and Martens 1978). These studies present good evidence that while uptake at lower concentrations be concentration dependent (Edwards et al. 1982), it is unlikely that this is true at higher concentrations. The data presented in these bioaccumulation studies suggest that a maximum uptake rate is reached, possibly an upper limit of the solubility of the PAH in soil water. Edwards (1983) draws a

Table 3.5.2: Bioaccumulation of PBBs in Higher Plants

Chemical	Test Organism	Life Stage or Size	Method	Response Variable	Tissue Concentration ($\mu\text{g/g}$)	Exposure Concentration ($\mu\text{g/kg}$)	Exposure Duration	Reference
Anthracene	Cabbage	Mature plant	A	Leaf tissue concentration	trace to 10	unknown	seasonal	Pyssalo, 1979
	Celery	Mature plant	A	Tissue concentration	trace to 5	unknown	seasonal	Pyssalo, 1979
	Cucumber	Mature plant	A	Leaf tissue concentration	nd*	unknown	seasonal	Pyssalo, 1979
	Fall	Mature plant	A	Leaf tissue concentration	0.7	unknown	seasonal	Kresch, et al., 1981
	Lettuce	Mature plant	A	Leaf tissue concentration	0.07	unknown	seasonal	Kresch, et al., 1981
	Lettuce	Mature plant	A	Leaf tissue concentration	nd to 1	unknown	seasonal	Pyssalo, 1979
	Parsley	Mature plant	A	Leaf tissue concentration	nd to trace	unknown	seasonal	Pyssalo, 1979
	Red pepper	Mature plant	A	Leaf tissue concentration	1	unknown	seasonal	Pyssalo, 1979
	Tansy	Mature plant	A	Root tissue concentration	170	unknown	seasonal	Guddal, 1979
	Tomato	Mature plant	A	Leaf tissue concentration	1	unknown	seasonal	Pyssalo, 1979
Benzanthracene	Cabbage	Mature plant	A	Leaf tissue concentration	24	unknown	seasonal	Pyssalo, 1979
	Celery	Mature plant	A	Tissue concentration	16	unknown	seasonal	Pyssalo, 1979
	Cucumber	Mature plant	A	Leaf tissue concentration	trace	unknown	seasonal	Pyssalo, 1979
	Fruit	Marketed produce	A	Tissue concentration	0.11	unknown	seasonal	Dennis et al., 1983
	Leafy vegetables	Marketed produce	A	Tissue concentration	0.15	unknown	seasonal	Dennis et al., 1983

Table 3.5.2: Bioaccumulation of PMs in Higher Plants (cont'd)

Chemical	Test Organism	Life Stage or Size	Method	Response Variable	Tissue Concentration ($\mu\text{g}/\text{g}^{\text{dw}}$)	Exposure Concentration ($\mu\text{g}/\text{g}^{\text{dw}}$)	Exposure Duration	Reference
Benz(a)anthracene (Cont'd)	Lettuce	Mature plant	A	Leaf tissue concentration	0.2	unknown	seasonal	Everseth, et al., 1981
	Lettuce	Mature plant	A	Leaf tissue concentration	25	unknown	seasonal	Pyssalo, 1979
	Parsley	Mature plant	A	Leaf tissue concentration	nd	unknown	seasonal	Pyssalo, 1979
	Red pepper	Mature plant	A	Leaf tissue concentration	nd	unknown	seasonal	Pyssalo, 1979
	Root vegetable produce	Mature plant	A	Tissue concentration	0.05	unknown	seasonal	Dennis, et al., 1983
Benz(a)anthracene (/Dibenz(a,h)anthracene)	Tomato	Mature plant	A	Leaf tissue concentration	nd	unknown	seasonal	Pyssalo, 1979
	Carrot	Mature plant	A	Leaf tissue concentration	25 to 41.2 (fw)	21 to 6540 dw	seasonal	Linne and Martens, 1978
	Carrot	Mature plant	A	Root tissue concentration	1.3 to 32.3 (fw)	21 to 6640 dw	seasonal	Linne and Martens, 1978
	Mushrooms	Mature sporophytes	A	Tissue concentration	nd	21 to 6640 dw	seasonal	Linne and Martens, 1978
	Fruit	Marketed produce	A	Tissue concentration	0.06	unknown	seasonal	Dennis, et al., 1983
Benz(b)fluoranthene	Leafy vegetables	Marketed produce	A	Tissue concentration	0.16	unknown	seasonal	Dennis, et al., 1983
	Lettuce	Mature plants	A	Tissue concentration	0.6	unknown	seasonal	Everseth, et al., 1981
	Root vegetables	Marketed produce	A	Tissue concentration	0.03	unknown	seasonal	Dennis, et al., 1983
	Carrot	Mature plants	AS	Leaf tissue concentration (fw)	12.5 to 41.7	20 to 6650 dw	seasonal	Linne and Martens, 1978
	Carrot	Mature plants	AS	Root tissue concentration (fw)	5.8 to 41.2	20 to 6650 dw	seasonal	Linne and Martens, 1978

Table 3.5.2: Bioaccumulation of PAHs in Higher Plants (cont'd)

Chemical	Test Organism	Life Stage or Site	Method	Response Variable	Tissue Concentration ($\mu\text{g}/\text{kg}^{\text{w}}$)	Exposure Concentration ($\mu\text{g}/\text{kg}^{\text{w}}$)	Exposure Duration	Reference
Benzo(ghi)perylene (Cont'd)	Cotiler trees	Litter	A	Tissue concentration	51	unknown	life long	Thomas, et al., 1984
	<i>Corylus avellana</i>	Mature plants	A	Tissue concentration	1	unknown	life long	Thomas, et al., 1984
	Deciduous trees	Litter	A	Tissue concentration	24	unknown	life long	Thomas, et al., 1984
	<i>Dischadonia fluquosa</i>	Mature plants	A	Tissue concentration	2	unknown	life long	Thomas, et al., 1984
	<i>Junioerus communis</i>	Mature plants	A	Needle tissue concentration	1	unknown	life long	Thomas, et al., 1984
	Mushroom	Mature sporophyte	BS, A	Tissue concentration	nd	6 to 1540	seasonal	Lone s/o Martens, 1972
	Oat	Mature plants	BS, A	Grain tissue concentration	26.6	9.3 to 27.5	seasonal	Ellwardt, 1977
	Oat	Mature plants	BS, A	Straw tissue concentration	17.7	9.3 to 27.5	seasonal	Ellwardt, 1977
	<i>Pinus sylvestris</i>	Mature plants	A	First year needle tissue concentration	1	unknown	life long	Thomas, et al., 1984
	<i>Pinus sylvestris</i>	Mature plants	A	Second year needle tissue concentration	1	unknown	life long	Thomas, et al., 1984
	Potato	Mature plants	A	Tuber tissue concentration	5.0	unknown	seasonal	Ellwardt, 1977
	Potato	Mature plants	BS, A	Haule tissue concentration	11.8	9.3 to 27.5	seasonal	Ellwardt, 1977
	Rye	Mature plants	BS, A	Grain tissue concentration	9.7	9.3 to 27.5	seasonal	Ellwardt, 1977
	Rye	Mature plants	BS, A	Straw tissue concentration	6.8	9.3 to 27.5	seasonal	Ellwardt, 1977
	<i>Vaccinium myrtillus</i>	Mature plants	A	Tissue concentration	3	unknown	life long	Thomas, et al., 1984

Table 3.5.2: Bioaccumulation of PAHs in Higher Plants (cont'd)

Chemical	Test Organism	Life Stage or Site	Method	Response Variable	Tissue Concentration ($\mu\text{g/g}$)	Exposure Concentration ($\mu\text{g/l}$)	Exposure Duration	Reference
Benz(a)pyrene	Bean	Mature plants	A	Tissue concentration	1.0 to 6.7	0 to 15000	seasonal	Pritt, 1983
	<i>Betula verrucosa</i>	Mature plants	A	Leaf tissue concentration	1	unknown	life long	Thomas, et al., 1984
	<i>B. verrucosa</i>	Mature plants	A	Bark tissue concentration	2	unknown	life long	Thomas, et al., 1984
	Cabbage	Mature plants	A	Tissue concentration	1	unknown	seasonal	Pysalo, 1975
	Cantaloupe	Mature plants	BL, L	Fruit tissue concentration	nd	saturation () .1	12 weeks	Blum and Swarbuch, 1977
	Carrot	Mature plant	A	Leaf tissue concentration	1.1 to 4.3	0.085 to 0.185 $\mu\text{g/l}$	80 days	Steffris, 1975
	Carrot	Mature plant	A	Leaf tissue concentration	1 to 30	0 to 4000	17.5 weeks	Muller, 1976
	Carrot	Mature plant	A	Root tissue concentration	0.08 to 0.19	0.085 to 0.185 $\mu\text{g/l}$	80 days	Steffris, 1975
	Carrot	Mature plant	A	Root tissue concentration	0 to 330	0 to 4000	17.5 weeks	Muller, 1976
	Carrot	Mature plants	BS	Leaf tissue concentration (fw)	3.1 to 11.2	5 to 1430	seasonal	Line and Martens, 1978
	Carrot	Mature plants	BS	Root tissue concentration (fw)	nd to 18.8	5 to 1430	seasonal	Line and Martens, 1978
	Carrot	Mature plants	A	Tissue concentration	0.07 to 9.7	0.5 to 15000	seasonal	Pritt, 1983
	Celery	Mature plants	A	Tissue concentration	nd	unknown	seasonal	Pysalo, 1975
	Conifer trees	Litter	A	Tissue concentration	42	unknown	life long	Thomas, et al., 1984
	Corn	Oil	A	Concentration	0.7	unknown	life long	Howard and Fazio, 1969
	<i>Curtius americana</i>	Mature plants	A	Tissue concentration	1	unknown	life long	Thomas, et al., 1984

Table 3.5.2: Bioaccumulation of PBs in Higher Plants (cont'd)

Chemical	Test Organism	Life Stage or Size	Method	Response Variable	Tissue Concentration ($\mu\text{g}/\text{kg}^{\text{d}}$)	Exposure Concentration ($\mu\text{g}/\text{kg}^{\text{d}}$)	Exposure Duration	Reference
Benzolipyrrene (Cont'd)	Cottonseed	Mature plants	HS, L	Tissue concentration	nd	saturation (> 4)	22 weeks	Blum and Starbuck, 1977
	Cottonseed	Oil	A	Concentration	0.4	unknown	life long	Howard and Hartz, 1969
	Cucumber	Mature plants	A	Tissue concentration	nd	unknown	seasonal	Pysalo, 1975
	Deciduous trees	Litter	A	Tissue concentration	55	unknown	life long	Thomas, et al., 1984
	<i>Onoclasia fluvosa</i>	Mature plants	A	Tissue concentration	-	unknown	life long	Thomas, et al., 1984
	Fruit	Marketed produce	A	Tissue concentration	0.07	unknown	seasonal	Dennis, et al., 1984
	Green bean	Mature plants	GL, L	Fruit, stem and leaf tissue	nd	saturation (> 4)	6 weeks	Blum and Starbuck, 1977
	<i>Juniperus communis</i>	Mature plants	A	Needle tissue concentration	1	unknown	life long	Thomas, et al., 1984
	Leafy vegetables	Marketed produce	A	Tissue concentration	0.09	unknown	seasonal	Dennis, et al., 1984
	Lettuce	Mature plant	A	Leaf tissue concentration	0.2 to 1.3	0.085 to 0.185 $\mu\text{g}/\text{g}$	unknown	Steffried, 1975
	Lettuce	Mature plants	A	Tissue concentration	4	unknown	seasonal	Pysalo, 1979
	Lettuce	Mature plants	HS, A	Tissue concentration	0.21 to 10.85	0.5 to 15000	seasonal	Fritz, 1983
	Lettuce	Mature plants	A	Tissue concentration	0.2	unknown	seasonal	Keseth, et al., 1981
	Mushroom	Mature sporophytic	HS, A	Tissue concentration	nd	6 to 1540	seasonal	Linn and Hartens, 1978

Table 3.5.2: Bioaccumulation of PAHs in Higher Plants (cont'd)

Chemical	Test Organism	Life Stage or Size	Method	Response Variable	Tissue Concentration ($\mu\text{g/g}^d$)	Exposure Concentration ($\mu\text{g/kg}^d$)	Exposure Duration	Reference
Benzo(a)pyrene (Cont'd)	Oat	Mature plants	BS, A	Grain tissue concentration	1-5	23.2 to 85.2	seasonal	Ellwardt, 1977
	Oat	Mature plants	BS, A	Straw tissue concentration	8-2	23.2 to 85.2	seasonal	Ellwardt, 1977
	Olive	Oil	A	Concentration	0-5	unknown	life long	Howard and Fazio, 1963
	Parsley	Mature plants	A	Tissue concentration	nd	unknown	seasonal	Pysyalo, 1979
	Parsley	Mature plants	A	Tissue concentration	0.56 to 78.3	0 to 15000	seasonal	Fritze, 1983
	Pea	Mature plants	A	Tissue concentration	0.62 to 9.6	0 to 15000	seasonal	Fritze, 1983
	Peanut	Oil	A	Concentration	0-4	unknown	life long	Howard and Fazio, 1963
	Pepper (red)	Mature plants	A	leaf tissue concentration	nd	unknown	seasonal	Pysyalo, 1979
	Pinus sylvestris	Mature plants	A	First year needle tissue concentration	1	unknown	life long	Thomas, et al., 1984
	Pinus sylvestris	Mature plants	A	Second year needle tissue concentration	1	unknown	life long	Thomas, et al., 1984
	Potato	Mature plants	A	Tuber tissue concentration	0.8	23.2 to 85.2	seasonal	Ellwardt, 1977
	Potato	Mature plants	BS, A	Tuber tissue concentration	0.11 to 23.4	14 to 176	seasonal	Shand and Cohan, 1972
	Potato	Mature plants	BS, A	Haula tissue concentration	8.4	23.2 to 85.2	seasonal	Ellwardt, 1977
	Potato	Mature plants	A	Peel tissue concentration	6.47 to 40.4	0 to 15006	seasonal	Fritze, 1983

Table 3.5.2: Bioaccumulation of PBBs in Higher Plants (cont'd)

Chemical	Test Organism	Life Stage or Site	Method	Response Variable	Tissue Concentration ($\mu\text{g/g}$)	Exposure Concentration ($\mu\text{g/g}$)	Exposure Duration	Reference
Benzodipyrone (Cont'd)	Potato	Mature plants	A	Flesh tissue concentration	0.06 to 0.26	0 to 15000	seasonal	Fritze, 1983
	Radish	Mature	A	Tissue	0.48 to 7.65	0 to 15000	seasonal	Fritze, 1983
	Radish	Mature plant	GS ^a	Leaf tissue concentration	0 to 80	0 to 4000	7 weeks	Muller, 1976
	Radish	Mature plant	GS	Root tissue concentration	0 to 35	0 to 4000	7 weeks	Muller, 1976
	Root vegetables	Marketed produce	A	Tissue concentration	0.06	unknown	seasonal	Genns, et al., 1983
	Gye	Mature plants	GS, A	Grain tissue concentration	2.7	23.2 to 89.2	seasonal	Ellwardt, 1977
	Gye	Mature plants	GS, A	Straw tissue concentration	3.3	23.2 to 89.2	seasonal	Ellwardt, 1977
	Gye	Mature plants	GS, A	Seed tissue concentration	.35 to .35	14 to 170 μg	seasonal	Shabad and Cohen, 1972
	Gye	Young plants	GL ^a	Leaf tissue concentration	2.76 to 9.14	165 to 1650 $\mu\text{g/L}$	2 to 30 days	Burr, 1970
	Gye	Young plants	GS	Leaf tissue concentration	0.0 to 0.2	3.3 $\mu\text{g/g}$ μg	6 to 30 days	Orr, 1970
	Soybean	Oil	A	Concentration	1.4	unknown	seasonal	Howard and Fazio, 1969
Benzodipyrone (Cont'd)	Spruce	Mature plants	A	Tissue concentration	0.43 to 25.1	0 to 15000	seasonal	Fritze, 1983
	Toato	Mature plants	A	Leaf tissue concentration	nd	unknown	seasonal	Frisalo, 1979
	Toato	Mature plants	GS, A	Tissue concentration	0.08 to 0.5	0 to 15000	seasonal	Fritze, 1983
	Vaccinium myrtillus	Mature plants	A	Tissue concentration	1	unknown	life long	Thomas, et al., 1984
	Wheat (spring)	Mature plants	GS, A	Seed and straw tissue concentration	.38 to 26.7	14 to 170 μg	seasonal	Shabad and Cohen, 1972

Table 3.5.2: Bioaccumulation of PBBs in Higher Plants (cont'd)

Chemical	Test Organism	Life Stage or Site	Method	Response Variable	Tissue Concentration ($\mu\text{g/g}$)	Exposure Concentration ($\mu\text{g/kg}$)	Exposure Duration	Reference
Benzo(a)pyrene	Cabbage	Mature plants	A	Tissue concentration	15	unknown	seasonal	Pyssalo, 1979
	Carrot	Mature plants	BS, A	Leaf tissue concentration	5.9 to 27.7	9 to 2710	seasonal	Linne and Martens, 1978
	Carrot	Mature plants	BS, A	Root tissue concentration	0.5 to 41.2	9 to 2710	seasonal	Linne and Martens, 1978
	Celery	Mature plants	A	Tissue concentration	nd	unknown	seasonal	Pyssalo, 1979
	Cucumber	Mature plants	A	Leaf tissue concentration	nd	unknown	seasonal	Pyssalo, 1979
	Fruit	Marketed produce	A	Tissue concentration	0.03	unknown	seasonal	Dennis, et al., 1982
	Leafy vegetables	Marketed produce	A	Tissue concentration	0.10	unknown	seasonal	Dennis, et al., 1983
	Lettuce	Mature plants	A	Tissue concentration	10	unknown	seasonal	Pyssalo, 1979
	Lettuce	Mature plants	A	Tissue concentration	0.4	unknown	seasonal	Eweath et al., 1981
	Mushrooms	Mature sporophytes	BS, A	Tissue concentration	nd	9 to 2710	seasonal	Linne and Martens, 1978
	Oat	Mature plants	BS, A	Grain tissue concentration	7.5	48.3 to 88.0	seasonal	Ellwardt, 1977
	Oat	Mature plants	BS, A	Straw tissue concentration	15.2	48.3 to 88.0	seasonal	Ellwardt, 1977
	Rasperry	Mature plants	A	Tissue concentration	nd	unknown	seasonal	Pyssalo, 1979
	Pepper (red)	Mature plants	A	Leaf tissue concentration	5	unknown	seasonal	Pyssalo, 1979
	Potato	Mature plants	BS, A	Tuber tissue concentration	2.6	48.3 to 88.0	seasonal	Ellwardt, 1977
	Potato	Mature plants	BS, A	Whole tissue concentration	33	48.3 to 88.0	seasonal	Ellwardt, 1977

Table 3.5.2: Bioaccumulation of PMHs in Higher Plants (cont'd)

Chemical	Test Organism	Life Stage or Size	Method	Response Variable	Tissue Concentration ($\mu\text{g}/\text{kg}^w$)	Exposure Concentration ($\mu\text{g}/\text{kg}^w$)	Exposure Duration	Reference
Benzo(a)pyrene (Cont'd)	Root vegetables	Marketed produce	A	Tissue concentration	0.02	unknown	seasonal	Dennis et al., 1983
	Eye	Mature plants	BS, A	Grain tissue concentration	5.5	48.3 to 88.0	seasonal	Ellwardt, 1977
	Eye	Mature plants	BS, A	Straw tissue concentration	3.5	48.3 to 88.0	seasonal	Ellwardt, 1977
	Tomato	Mature plants	A	Leaf tissue concentration	nd	unknown	seasonal	Pysyalo, 1979
Chrysene	Fruit	Marketed produce	A	Tissue concentration	0.23	unknown	seasonal	Dennis et al., 1983
	Leafy vegetables	Marketed produce	A	Tissue concentration	0.33	unknown	seasonal	Dennis et al., 1983
	Root vegetables	Marketed produce	A	Tissue concentration	0.23	unknown	seasonal	Dennis et al., 1983
	Cabbage	Mature plants	A	Tissue concentration	35	unknown	seasonal	Pysyalo, 1979
Chrysene/Tripicylene	Celery	Mature plants	A	Tissue concentration	16	unknown	seasonal	Pysyalo, 1979
	Cucumber	Mature plants	A	Leaf tissue concentration	nd	unknown	seasonal	Pysyalo, 1979
	Kale	Mature plants	A	Tissue concentration	nd	unknown	seasonal	Kveteb et al., 1981
	Lettuce	Mature plants	A	Tissue concentration	10	unknown	seasonal	Pysyalo, 1979
	Lettuce	Mature plants	A	Tissue concentration	1.0	unknown	seasonal	Kveteb et al., 1981
	Parsley	Mature plants	A	Tissue concentration	21	unknown	seasonal	Pysyalo, 1979
	Pepper (red)	Mature plants	A	Leaf tissue concentration	nd	unknown	seasonal	Pysyalo, 1979
	Tomato	Mature plants	A	Leaf tissue concentration	nd	unknown	seasonal	Pysyalo, 1979

Table 3.5.2. Bioaccumulation of PMAs in Higher Plants (cont'd)

Chemical	Test Organism	Life Stage or Size	Method	Response Variable	Tissue Concentration ($\mu\text{g/g}^d$)	Exposure Concentration ($\mu\text{g/g}^d$)	Exposure Duration	Reference
Dibenz[ah]anthracene	Oat	Mature plants	BS, A	Grain tissue concentration	10.3	38.1 to 83.4	seasonal	Ellwardt, 1977
	Oat	Mature plants	BS, A	Straw tissue concentration	13.3	38.1 to 83.4	seasonal	Ellwardt, 1977
	Potato	Mature plants	BS, A	Tuber tissue concentration	nd	38.1 to 63.4	seasonal	Ellwardt, 1977
	Potato	Mature plants	BS, A	Haule tissue concentration	8.8	38.1 to 63.4	seasonal	Ellwardt, 1977
	Barley	Mature plants	BS, A	Grain tissue concentration	4.1	38.1 to 63.4	seasonal	Ellwardt, 1977
	Barley	Mature plants	BS, A	Grain tissue concentration	3.6	38.1 to 63.4	seasonal	Ellwardt, 1977
	Fruit	Marketed produce	A	Tissue concentration	0.51	unknown	seasonal	Dennis et al., 1983
	Leafy vegetables	Marketed produce	A	Tissue concentration	0.01	unknown	seasonal	Dennis et al., 1983
	Oat	Mature plants	BS, A	Grain tissue concentration	20.2	17.1 to 29.6	seasonal	Ellwardt, 1977
	Oat	Mature plants	BS, A	Straw tissue concentration	5.10	17.1 to 29.6	seasonal	Ellwardt, 1977
Dibenz[ah]acanthracene	Potato	Mature plants	BS, A	Tuber tissue concentration	5.0	17.1 to 29.6	seasonal	Ellwardt, 1977
	Potato	Mature plants	BS, A	Haule tissue concentration	11.0	17.1 to 29.6	seasonal	Ellwardt, 1977
	Root vegetables	Marketed produce	A	Tissue concentration	nd	unknown	seasonal	Dennis et al., 1983
	Barley	Mature plants	BS, A	Grain tissue concentration	1.3	17.1 to 29.6	seasonal	Ellwardt, 1977
	Barley	Mature plants	BS, A	Grain tissue concentration	3.4	17.1 to 29.6	seasonal	Ellwardt, 1977
	Carrot	Mature plants	BS, A	Leaf tissue concentration	nd to 5.0 (μg)	2 to 660	seasonal	Line and Marzans, 1978

Table 3.5.2: Bioaccumulation of PAHs in Higher Plants (cont'd)

Chemical	Test Organism	Life Stage or Site	Method	Response Variable	Tissue Concentration ($\mu\text{g/g}$)	Exposure Concentration ($\mu\text{g/g}$)	Exposure Duration	Reference
Oithonolob/ae] anthracene	Carrot	Mature plants	BS, A	Root tissue concentration	nd to 1.3	2 to 660	seasonal	Lione and Martens, 1978
	Mushroom	Mature sporophyte	BS, A	Tissue concentration	nd	2 to 660	seasonal	Lione and Martens, 1978
Gibberol[anthracene	Carrot	Mature plants	BS, A	Leaf tissue concentration	0.6 to 6.3	3 to 740	seasonal	Lione and Martens, 1978
	Carrot	Mature plants	BS, A	Root tissue concentration	nd to 5.7	3 to 740	seasonal	Lione and Martens, 1978
Fluoranthene	Mushroom	Mature sporophyte	BS, A	Tissue concentration	nd	3 to 740	seasonal	Lione and Martens, 1978
	Betula verrucosa	Mature plants	A	Leaf tissue concentration	1.0	unknown	lifelong	Thomas et al., 1984
B. verrucosa	Mature plants	Mature plants	A	Bark tissue concentration	37	unknown	lifelong	Thomas et al., 1984
	Cabbage	Mature plants	A	Tissue concentration	26	unknown	seasonal	Pysalo, 1979
Celery	Mature plants	Mature plants	A	Tissue concentration	20	unknown	seasonal	Pysalo, 1979
	Coniferous plants	Litter	A	Tissue concentration	162	unknown	lifelong	Thomas et al., 1984
Corflus avellana	Mature plants	Mature plants	A	Tissue concentration	22	unknown	lifelong	Thomas et al., 1984
	Cucumber	Mature plants	A	Leaf tissue concentration	nd	unknown	seasonal	Pysalo, 1979
Deciduous plants	Litter	Litter	A	Tissue concentration	120	unknown	lifelong	Thomas et al., 1984
	Deschampsia flexuosa	Mature plants	A	Tissue concentration	8	unknown	lifelong	Thomas et al., 1984
Fruit	Marketed produce	Marketed produce	A	Tissue concentration	0.93	unknown	seasonal	Genies et al., 1983
	Juniperus communis	Mature plants	A	Tissue concentration	8	unknown	lifelong	Thomas et al., 1984

Table 3.5.2: Bioaccumulation of PHA in Higher Plants (cont'd)

Chemical	Test Organism	Life Stage or Size	Method	Response Variable	Tissue Concentration ($\mu\text{g}/\text{kg}^w$)	Exposure Concentration ($\mu\text{g}/\text{kg}^w$)	Exposure Duration	Reference
Fluoranthene (cont'd)	Kale	Mature plants	A	Tissue concentration	7.6	unknown	seasonal	Kveseth et al., 1981
	Leafy vegetables	Marketed produce	A	Tissue concentration	1.5	unknown	seasonal	Dennis et al., 1983
	Lettuce	Mature plants	A	Tissue concentration	1.3	unknown	seasonal	Kveseth et al., 1981
	Lettuce	Mature plants	A	Tissue concentration	25	unknown	seasonal	Pysalo, 1979
	Parsley	Mature plants	A	Tissue concentration	15	unknown	seasonal	Pysalo, 1979
	Pepper (red)	Mature plants	A	Leaf tissue concentration	5	unknown	seasonal	Pysalo, 1979
	<i>Pinus sylvestris</i>	Mature plants	A	First year needle tissue concentration	10	unknown	lifelong	Thomas et al., 1984
	<i>Pinus sylvestris</i>	Mature plants	A	Second year needle tissue concentration	10	unknown	lifelong	Thomas et al., 1984
	Root vegetables	Marketed produce	A	Tissue concentration	0.26	unknown	seasonal	Dennis et al., 1983
	Tansy	Mature plants	A	Root tissue concentration	nd	unknown	lifelong	Guddal, 1959
Fluorene	Tomato	Mature plants	A	Leaf tissue concentration	5	unknown	seasonal	Pysalo, 1979
	Gale	Mature plants	A	Tissue concentration	8.0	unknown	seasonal	Kveseth et al., 1981
	Lettuce	Mature plants	A	Tissue concentration	nd	unknown	seasonal	Kveseth et al., 1981
Indeno(1,2,3-cd)pyrene	<i>Betula verrucosa</i>	Mature plants	A	Leaf tissue concentration	0	unknown	lifelong	Thomas et al., 1984
	<i>B. verrucosa</i>	Mature plants	A	Bark tissue concentration	2.0	unknown	lifelong	Thomas et al., 1984

Table 3.5.2: Bioaccumulation of PMs in Higher Plants (cont'd)

Chemical	Test Organism	Life Stage or Size	Method	Response Variable	Tissue Concentration ($\mu\text{g/g}$)	Exposure Concentration ($\mu\text{g}/\mu\text{g}$)	Exposure Duration	Reference
Indeno[1,2,3-cd]perylene (Cont'd)	Coniferous plants	Litter	A	Tissue concentration	67	unknown	lifelong	Thomas et al., 1984
	<i>Corylus avellana</i>	Mature plants	A	Tissue concentration	1.0	unknown	lifelong	Thomas et al., 1984
	Deciduous plants	Litter	A	Tissue concentration	59	unknown	lifelong	Thomas et al., 1984
	<i>Deschampsia flexuosa</i>	Mature plants	A	Tissue concentration	5	unknown	lifelong	Thomas et al., 1984
	<i>Juniperus communis</i>	Mature plants	A	Tissue concentration	1	unknown	lifelong	Thomas et al., 1984
	<i>Pinus sylvestris</i>	Mature plants	A	First year needle tissue concentration	2	unknown	lifelong	Thomas et al., 1984
	<i>Pinus sylvestris</i>	Mature plants	A	Second year needle tissue concentration	1	unknown	lifelong	Thomas et al., 1984
Indeno[1,2,3-cd]pyrene	Carrot	Mature plants	BS, A	Leaf tissue concentration (fw)	1.8 to 12.4	6 to 1450	seasonal	Linne and Martens, 1978
	Carrot	Mature plants	BS, A	Root tissue concentration (fw)	nd to 10.3	6 to 1450	seasonal	Linne and Martens, 1978
	Fruit	Marketed produce	A	Tissue concentration	nd	unknown	seasonal	Dennis et al., 1983
	Leafy vegetables	Marketed produce	A	Tissue concentration	nd	unknown	seasonal	Dennis et al., 1983
	Mushroom	Mature sporocystic	BS, A	Tissue concentration	nd	6 to 1450	seasonal	Linne and Martens, 1978
	Oat	Mature plants	BS, A	Grain tissue concentration	5.4	unknown	seasonal	Ellwardt, 1977
	Oat	Mature plants	BS, A	Straw tissue concentration	nd	unknown	seasonal	Ellwardt, 1977
	Potato	Mature plants	BS, A	Tuber concentration	8.0	unknown	seasonal	Ellwardt, 1977

Table 3.5.2: Bioaccumulation of PMs in Higher Plants (cont'd)

Chemical	Test Organism	Life Stage or Size	Method	Response Variable	Tissue Concentration ($\mu\text{g}/\text{kg}^{\text{w}}$)	Exposure Concentration ($\mu\text{g}/\text{kg}^{\text{w}}$)	Exposure Duration	Reference
Indeno[1,2,3-cd]pyrene (Cont'd)	Potato	Mature plants	BS, A	Harle concentration	5.6	unknown	seasonal	Ellwardt, 1977
	Root vegetables	Marketed produce	A	Tissue concentration	nd	unknown	seasonal	Dennis et al., 1983
	Barley	Mature plants	BS, A	Grain tissue concentration	nd	unknown	seasonal	Ellwardt, 1977
	Barley	Mature plants	BS, A	Straw tissue concentration	1.3	unknown	seasonal	Ellwardt, 1977
1-methylphenanthrene/Methylanthracene	Bale	Mature plants	A	Tissue concentration	5.8	unknown	seasonal	Gveseth et al., 1981
	Lettuce	Mature plants	A	Tissue concentration	6.3	unknown	seasonal	Gveseth et al., 1981
	Cabbage	Mature plants	A	Tissue concentration	31	unknown	seasonal	Pyssalo, 1979
Perylene	Carrot	Mature plants	BS, A	Leaf tissue concentration (fw)	nd to 1.3	0.5 to 280	seasonal	Linne and Martens, 1978
	Carrot	Mature plants	BS, A	Root tissue concentration (fw)	nd to 3.0	0.5 to 280	seasonal	Linne and Martens, 1978
	Celery	Mature plants	A	Tissue concentration	30	unknown	seasonal	Pyssalo, 1979
Cucurbit	Cucumber	Mature plants	A	Leaf tissue concentration	nd	unknown	seasonal	Pyssalo, 1979
	Lettuce	Mature plants	A	Tissue concentration	nd	unknown	seasonal	Gveseth et al., 1981
	Lettuce	Mature plants	A	Tissue concentration	15	unknown	seasonal	Pyssalo, 1979
Mushroom	Mushroom	Mature sporophyte	BS, A	Tissue concentration	nd	0.5 to 280	seasonal	Linne and Martens, 1978
	Oat	Mature plants	BS, A	Grain tissue concentration	13.1	4.2 to 7.2	seasonal	Ellwardt, 1977
	Oat	Mature plants	BS, A	Straw tissue concentration	9.3	4.2 to 7.2	seasonal	Ellwardt, 1977

Table 3.5.2: Bioaccumulation of PAHs in Higher Plants (Cont'd)

Chemical	Test Organism	Life Stage or Size	Method	Response Variable	Tissue Concentration ($\mu\text{g/g dw}$)	Exposure Concentration ($\mu\text{g/l}$)	Exposure Duration	Reference
Perylene (Cont'd)	Parsley	Mature plants	A	Tissue concentration	nd	unknown	seasonal	Pysalo, 1979
	Pepper (red)	Mature plants	A	Leaf tissue concentration	5	unknown	seasonal	Pysalo, 1979
	Potato	Mature plants	BS, A	Tuber tissue concentration	6.2	4.2 to 7.2	seasonal	Ellwardt, 1977
	Potato	Mature plants	BS, A	Walls tissue concentration	6.8	4.2 to 7.2	seasonal	Ellwardt, 1977
	Bye	Mature plants	BS, A	Grain tissue concentration	9.9	4.2 to 7.2	seasonal	Ellwardt, 1977
	Bye	Mature plants	BS, A	Straw tissue concentration	4.8	4.2 to 7.2	seasonal	Ellwardt, 1977
	Tomato	Mature plants	A	Leaf tissue concentration	nd	unknown	seasonal	Pysalo, 1979
	Cabbage	Mature plants	A	Tissue concentration	34	unknown	seasonal	Pysalo, 1979
	Celery	Mature plants	A	Tissue concentration	21	unknown	seasonal	Pysalo, 1979
	Cucumber	Mature plants	A	Leaf tissue concentration	nd	unknown	seasonal	Pysalo, 1979
Phenanthrene	Kale	Mature plants	A	Tissue concentration	16.9	unknown	seasonal	Keseth et al., 1981
	Lettuce	Mature plants	A	Tissue concentration	4.4	unknown	seasonal	Keseth et al., 1981
	Lettuce	Mature plants	A	Tissue concentration	13	unknown	seasonal	Pysalo, 1979
	Parsley	Mature plants	A	Tissue concentration	10	unknown	seasonal	Pysalo, 1979
	Pepper (red)	Mature plants	A	Leaf tissue concentration	1	unknown	seasonal	Pysalo, 1979
	Tomato	Mature plants	A	Leaf tissue concentration	nd	unknown	seasonal	Pysalo, 1979

Table 3.5.2: Bioaccumulation of PBBs in Higher Plants (cont'd)

Chemical	Test Organism	Life Stage or Site	Method	Response Variable	Tissue Concentration ($\mu\text{g}/\text{kg}^w$)	Exposure Concentration ($\mu\text{g}/\text{kg}^w$)	Exposure Duration	Reference
Pyrone	Cabbage	Mature plants	A	Tissue concentration	20	unknown	seasonal	Pysalo, 1979
	Delery	Mature plants	A	Tissue concentration	7	unknown	seasonal	Pysalo, 1979
	Cucumber	Mature plants	A	Leaf tissue concentration	nd	unknown	seasonal	Pysalo, 1979
	Fruit	Marketed produce	A	Tissue concentration	0.23	unknown	seasonal	Dennis et al., 1983
	Kale	Mature plants	A	Tissue concentration	4.2	unknown	seasonal	Gveseth et al., 1981
	Leafy vegetables	Marketed produce	A	Tissue concentration	1.23	unknown	seasonal	Dennis et al., 1983
	Lettuce	Mature plants	A	Tissue concentration	1.1	unknown	seasonal	Gveseth et al., 1981
	Lettuce	Mature plants	A	Tissue concentration	37	unknown	seasonal	Pysalo, 1979
	Parsley	Mature plants	A	Tissue concentration	nd	unknown	seasonal	Pysalo, 1979
	Pepper (red)	Mature plants	A	Leaf tissue concentration	nd	unknown	seasonal	Pysalo, 1979
	Beet vegetables	Marketed produce	A	Tissue concentration	0.24	unknown	seasonal	Dennis et al., 1983
	Tansy	Mature plants	A	Root tissue concentration	nd	unknown	life-long	Guddal, 1959
	Tomato	Mature plants	A	Leaf tissue concentration	5	unknown	seasonal	Pysalo, 1983

Footnotes: a - A: ambient (uncontrolled) exposure of roots and shoots

b - $\mu\text{g}/\text{kg}$ dry weight unless otherwise labelled

c - nd : non-detected

d - RS : root exposure via soil

e - RL : root exposure via liquid medium

f - RL, A : root exposure via soil plus ambient exposure

g - RL, L : root exposure via liquid medium, radiolabel

h - $\mu\text{g}/\text{kg}$ unless otherwise labelled

number of tentative conclusions about the concentration of PAH in plants. They are as follows:

- a) the PAH content of plants and soils is related to their proximity to a source,
- b) concentrations of PAH are usually greater for exterior surfaces of structures (such as fruit peel) than for interior tissues,
- c) aerial structures generally have higher PAH concentrations than below ground structures,
- d) broad leafed plants contain more PAH than narrow leafed plants on an absolute basis, due to their greater interaction with the atmosphere,
- e) most PAH contamination of vegetation is from atmospheric deposition,
- f) washing with water does not remove significant quantities of PAH from the surface of vegetables.

None of the studies in this section were regarded as primary data, basically because the concentration of PAH was determined only at the beginning of the experiment and was not measured again.

3.5.2.2.2 Metabolism and de novo Synthesis of PAH in Higher Plants

The literature in this section addresses the interaction of PAH and higher plants by examining translocation, metabolism and de novo synthesis (Table 3.5.3). It is most likely that transport of PAH through the plant is apoplastic (via the xylem) and is passive. Translocation may play an important role in modifying the expected effect of a PAH on plants, as rapid or absent translocation may modify

the concentration of the toxicant at a sensitive site. The primary or very good secondary studies in this section were Edwards et al. (1982), Edwards (1986) and Grimmer and Duvel (1970). The others were secondary due to uncertain exposure duration or concentration.

Edwards et al. (1982) compared the distribution of anthracene among leaf, stem and root in soybean plants which had been exposed to the PAH via the foliage (gaseous form) or roots (dissolved in hydroponic solution). This study demonstrated that most of the label (65 - 68%) was found in the source organs to which it had been applied (roots or leaves); less than 3% of the label was detected in the sink organs (stems and leaves, or stems and roots). The remaining label was found in the nutrient solution or supporting foam plug. A similar experiment with soybean in anthracene contaminated soil, demonstrated that much of the uptake into the roots was due to the artificially high availability of anthracene in hydroponic solution. Ninety three percent of the label remained in the soil (as compared to 12 to 28% remaining in solution in hydroponic exposure); less than 5% of the radiolabel was associated with the soybean, 2.3% of which was in the roots. This result is not surprising, as some PAH are known to adsorb to particulate matter of which soils may be an example, becoming unavailable for plant uptake. Edwards (1986) demonstrated a similar pattern of uptake and translocation in bushbean.

Edwards et al. (1982) also demonstrated that the uptake of anthracene was passive, (i.e. was taken up with the water stream into the xylem) and linearly related to concentration. The low level of translocation, however, suggests that once in the plant, the labeled compounds quickly bind to immobile cellular components, becoming unavailable to the transport system. Edwards (1986) demonstrated that the same pattern of label translocation was seen in bush bean, and that of the ^{14}C label in the tissue, more than 50% had been incorporated into non-extractable components (e.g. cell wall material), and only 15% could be retrieved as anthracene. In fact, there was very little anthracene found in leaves and stems of root-fed plants. It is likely that not anthracene, but its metabolites are

Table 3.3.3 : Metabolism and de novo Synthesis of PAHs in Higher Plants

Chemical	Test Organism	Life Stage or Site	Method	Response Variable	Effect	Exposure Concentration	Exposure Duration	Reference
Anthracene	Lettuce	From time of seeding	A ^a	Tissue ^a concentration	0.2 ug/kg	n/a	seasonal	Griener and Dovel 1970
	Beet	From time of seeding	A	Tissue concentration	0.1 ug/kg	n/a	seasonal	Griener and Dovel 1970
	Soybean	From time of seeding	A	Tissue concentration	trace	n/a	seasonal	Griener and Dovel 1970
	Tobacco	From time of seeding	A	Tissue concentration	0.0 ug/kg	n/a	seasonal	Griener and Dovel 1970
	Orange	From time of seeding	Fr. ^b	Tissue concentration	110 ug/kg	25 ug/L		Gutcher et al. 1967
Anthracene	Bush bean	14 days old	RL, L ^c	Uptake, translocation and metabolism	distribution of label; 60% root 2% stem 3% leaf 0.1% pod 1% solution 1% unaccounted (10% of label recovered as anthracene	0.01 ug/L	30 days	Edwards 1986
	Soybean	7 - 14 days old	RL, L	Uptake, translocation and metabolism	distribution of label; 55% root 2% stem 2% leaf	0.0025 ug/L	4 days	Edwards et al. 1982
Soybean	Soybean	7 - 14 days old	RS, L ^d	Uptake, translocation and metabolism	distribution of label; 91% soil 7% root	.01 ug/kg soil	4 days	Edwards et al. 1982

Table 3.5.3 Metabolism and de novo Synthesis of PMIs in Higher Plants (cont'd)

Chemical	Test Organism	Life Stage or Size	Method	Response Variable	Effect	Exposure Concentration	Exposure Duration	Reference
Anthracene (Cont'd)	Soybean	7 - 14 days old	PG, L	Uptake, translocation and metabolism	distribution of label: 69% leaf, 1.5% stem, 1.5% root	volatilization of anthracene, .005 ug/ml	3 days	Edwards et al., 1982
	Lettuce	From time of seeding	A	Tissue concentration	3.7 to 4.2 ug/kg	n/a	seasonal	Grimmer and Duvel, 1970
	Orange	Mature fruiting trees	FRL	Tissue concentration	110 ug/kg	250 ug/L		Gamber et al., 1967
	Eye	From time of seeding	A	Tissue concentration	0.64 to 1.6 ug/kg	n/a	seasonal	Grimmer and Duvel, 1970
Benzodipyrone	Soybean	From time of seeding	MS	Metabolism	metabolic products: 81aP-9,10-diol 85% 81aP-3-mono 15%		12 days	Kagisho et al., 1987
	Soybean	From time of seeding	A	Tissue concentration	3.1 ug/kg	n/a	seasonal	Grimmer and Duvel, 1970
	Tobacco	From time of seeding	A	Tissue concentration	1.8 ug/kg	n/a	seasonal	Grimmer and Duvel, 1970
	Lettuce	From time of seeding	A	Tissue concentration	4.2 to 4.3 ug/kg	n/a	seasonal	Grimmer and Duvel, 1970
Benzodipyrone	Eye	From time of seeding	A	Tissue concentration	1.2 to 3.4 ug/kg	n/a	seasonal	Grimmer and Duvel, 1970
	Soybean	From time of seeding	A	Tissue concentration	4.3 ug/kg	n/a	seasonal	Grimmer and Duvel, 1970
	Tobacco	From time of seeding	A	Tissue concentration	2.5 ug/kg	n/a	seasonal	Grimmer and Duvel, 1970
	Lettuce	From time of seeding	A	Tissue concentration	2.2 to 2.5 ug/kg	n/a	seasonal	Grimmer and Duvel, 1970
Benzodipyrone	Eye	From time of seeding	A	Tissue concentration	0.34 ug/kg	n/a	seasonal	Grimmer and Duvel, 1970
	Soybean	From time of seeding	A	Tissue concentration	1.5 ug/kg	n/a	seasonal	Grimmer and Duvel, 1970
	Tobacco	From time of seeding	A	Tissue concentration	1.1 ug/kg	n/a	seasonal	Grimmer and Duvel, 1970

Table 15.1 : Metabolism and de novo Synthesis of PAHs in Higher Plants (Cont'd)

Chemical	Test Organism	Life Stage or Site	Method	Response Variable	Effect	Exposure Concentration	Exposure Duration	Reference
Coronene	Lettuce	From tissue of seedling	A	Tissue concentration	0.5 to 0.7 $\mu\text{g/kg}$	n/a	seasonal	Griener and Duvel, 1970
	Eye	From tissue of seedling	A	Tissue concentration	0.1 $\mu\text{g/kg}$	n/a	seasonal	Griener and Duvel, 1970
	Soybean	From tissue of seedling	A	Tissue concentration	0.4 $\mu\text{g/kg}$	n/a	seasonal	Griener and Duvel, 1970
	Tobacco	From tissue of seedling	A	Tissue concentration	0.1 $\mu\text{g/kg}$	n/a	seasonal	Griener and Duvel, 1970
Dibenz(a,h)anthracene	Lettuce	From tissue of seedling	A	Tissue concentration	0.4 to 0.6 $\mu\text{g/kg}$	n/a	seasonal	Griener and Duvel, 1970
	Orange	Mature fruiting trees	FRL	Tissue concentration	1.1 $\mu\text{g/kg}$	250 $\mu\text{g/L}$		Griener and Duvel, 1970
								Gunter et al. 1967
	Eye	From tissue of seedling	A	Tissue concentration	0.1 $\mu\text{g/kg}$	n/a	seasonal	Griener and Duvel, 1970
Dibenz(a,h)pyrene	Soybean	From tissue of seedling	A	Tissue concentration	0.1 $\mu\text{g/kg}$	n/a	seasonal	Griener and Duvel, 1970
	Tobacco	From tissue of seedling	A	Tissue concentration	trace	n/a	seasonal	Griener and Duvel, 1970
	Orange	Mature fruiting trees	FRL	Tissue concentration	0.1 to 1.1 $\mu\text{g/kg}$	250 $\mu\text{g/L}$		Griener and Duvel, 1970
								Gunter et al. 1967
3-Methylcholanthrene	Orange	Mature fruiting trees	FRL	Tissue concentration	0.03 $\mu\text{g/kg}$	250 $\mu\text{g/L}$		Griener and Duvel, 1970
								Gunter et al. 1967
	Lettuce	From tissue of seedling	A	Tissue concentration	0.4 $\mu\text{g/kg}$	n/a	seasonal	Griener and Duvel, 1970
	Eye	From tissue of seedling	A	Tissue concentration	0.1 to 0.9 $\mu\text{g/kg}$	n/a	seasonal	Griener and Duvel, 1970
Benzo(a)pyrene	Soybean	From tissue of seedling	A	Tissue concentration	0.3 $\mu\text{g/kg}$	n/a	seasonal	Griener and Duvel, 1970
	Tobacco	From tissue of seedling	A	Tissue concentration	0.25 $\mu\text{g/kg}$	n/a	seasonal	Griener and Duvel, 1970
								Griener and Duvel, 1970
								Griener and Duvel, 1970

reductants: A - A : ambient exposure

B - FRL : front exposure to liquid

C - WL, L : root exposure to liquid solution, radiolabel

A - KS, L : root exposure to liquid solution, radiolabel

B - concentration of PAHs in tissue from plants grown to filtered air

F - not applicable, this study did not expose plants to PAH

translocated in the plant. The study by Durmishidze et al. (1974) reports wide variation in the proportioning of B[a]P between roots and leaves. Unfortunately, it is unclear from the paper, which data apply to foliar exposure and which data apply to root exposure; for this reason, it has not been included in the table.

A review by Edwards (1983) stated the following:

- a) some terrestrial plants can take up PAH through roots and/or leaves, and translocate them to distal plant parts,
- b) uptake rates are dependent on PAH concentrations, solubility, phase (vapor or particulate), molecular size, support media anchoring the plant, and plant species,
- c) PAH may concentrate in some plant parts more than others,
- d) some PAH may be metabolized by plants.

He concluded that although the results vary a great deal between experiments, the current data base suggests that PAH may enter the food chain via contamination of vegetation. Whether or not this poses a threat to consumers of the vegetation depends in part on degradation rates and products in air, soil and vegetation.

Negishi et al. (1987) analyzed the metabolites of benzo[a]pyrene in soybean leaves, and concluded that the soybean metabolized B[a]P using a mixed function oxidase, similarly to mammalian and eukaryotic systems. It was shown that the primary metabolite of B[a]P is B[a]P 9, 10 diol; the secondary metabolite is B[a]P 3 mono]. This is in contrast to Trenck and Sandermann (1980), who, using higher plant cell cultures, concluded that plants did not metabolize B[a]P similarly to mammalian systems.

Grimmer and Duvel (1970) conducted a rigorous investigation into the occurrence of de novo synthesis of PAH in various vegetable species. They concluded that de novo synthesis did not exist as no trace of six out of seven fairly common PAH was found in plants which had been grown in a controlled environment chamber, supplied with specially

filtered air. These plants were contrasted with individuals grown in the greenhouse and field, which did contain each of the seven PAH. Grimmer and Duvel (1970) concluded that these vegetable crops were incapable of de novo synthesis of PAH. This is in contrast to the observations of Borneff et al. (1968a, b), Payer et al. (1975) and Knutzen & Sortland (1982) who concluded that the opposite occurred in algae.

It is also in contrast to a review (Suess, 1976) which lists highly developed plants as endogenous synthesizers of PAH, along with phytoplankton, microorganisms and algae. Suess (1976) states that the endogenous synthesis of PAH forms a relatively small component of the global PAH load, and that these biosynthesized PAH tend to remain sequestered in their parent organism. Edwards (1983) suggests that much of the PAH content of oil or coal is derived from their original plant parent material.

3.6 HIGHER PLANT CELL CULTURE

3.6.1 Sublethal Toxicity

3.6.1.1 Subacute Effects

The studies described in this section concern the role of PAH as growth regulators, the first as a tool for manipulating cell culture (in combination with indole acetic acid, IAA) and the second for studying carcinogenic-like activity in plants (Table 3.6.1). None of these are primary studies, as they do not examine whole plants.

Benz[a]anthracene was shown to initiate differentiation of callus tissue, and B[a]A interacted with Indole Acetic Acid (IAA) in this effect. B[a]A tended to promote the formation of buds, whereas IAA tended to induce roots in callus cultures of tobacco. When both compounds were present at high concentrations, development of both root and shoot was inhibited (Kochkar and Sabharwal 1977). These results suggest that B[a]A may be useful in tissue culture work as a

Table 3.6.1: Higher Plant Cell Culture - Subacute Effects

Chemical	Test Organism	Life Stage or Size	Method	Response Variable	Effect	Exposure Concentration (mg/L)	Exposure Duration	Reference
Benzo(a)anthracene	Tobacco	Harbord callus	CL ^a	% of callus developing buds	81% develop about buds; 17% develop root buds	2.28	8 weeks	Kochlar and Saharwal, 1977
Benzo(a)pyrene	Carrot	Tap root meristematic callus	CL	Qualitative growth analysis	Growth was stimulated; some development was abnormal	1 to 150	unknown	Levine, 1976
Dibenz(a,h)anthracene	Carrot	Tap root meristematic callus	CL	Qualitative growth analysis	Growth was stimulated; some developmental was abnormal	1 to 150	unknown	Levine, 1976

Footnotes: a - CL = cells in liquid culture

Table 3.6.2: Toxicokinetics of Higher Plant Cell Cultures

Chemical	Test Organism	Life Stage or Size	Method	Response Variable	Effect	Exposure Concentration ($\mu\text{g/L}$)	Exposure Duration	Reference
Benz(a)l-pyrene	Atriplex hortensis	Actively dividing	CL L	Tissue concentration	90% of added label recovered as B(a)P. 15% recovered as metabolites	12.6	48 hours	Hares, 1983
	Chenopodium bonus-henricus	Actively dividing	CL L	Tissue concentration	83% of added label recovered as B(a)P. 15% recovered as metabolites	12.6	48 hours	Hares, 1983
	Chenopodium quinoa	Actively dividing	CL L	Tissue concentration	83% of added label recovered as B(a)P. 21% recovered as metabolites	12.6	48 hours	Hares, 1983
	Chenopodium rubrum	Actively dividing	CL L	Tissue concentration	10% of added label recovered as B(a)P. 15% recovered as metabolites	12.6	48 hours	Hares, 1983
Mung bean		Actively dividing	CL L	Tissue concentration	65% of cellular label recovered as B(a)P. 5% recovered as metabolites	12.6	48 hours	Hares, 1975
	Parsley	Actively dividing	CL L	Tissue concentration	?	0.0182 to 0.182	38 hours	Trench and Sandermann, 1978

Table 3.6.2: Toxicokinetics of Higher Plant Cell Cultures (Cont'd)

Chemical	Test Organism	Life Stage or Site	Method	Response Variable	Effect	Exposure Concentration ($\mu\text{M/L}$)	Exposure Duration	Reference
Benzolal- pyrene (Cont'd)	Parsley	Microsomes	CL L	Inhibition of cinnamic acid 4-hydroxylase	none	0.0146	1 hour	Trench and Sandermann, 1980
	Pea	Actively dividing	CL L	Tissue concentration	75% of cellular label recovered as BlalP, (2% recovered as metabolites	12.6	48 hours	Haras, 1977
	Pea	Microsomes	CL L	Tissue concentration	1.6-, 3.6- and 6.12- quinones were predominant metabolites	0.0146	1 hour	Trench and Sandermann, 1980
	Potato	Actively dividing	CL L	Tissue concentration	90% of cellular label recovered as BlalP, 1% recovered as metabolites	12.6	48 hours	Haras, 1977
Sorbuan	Sorbuan	Actively dividing	CL L	Tissue concentration	75% of cellular label recovered as BlalP, (2% recovered as metabolites	12.6	48 hours	Haras, 1977
	Sorbuan	Actively dividing	CL L	Tissue concentration	?	0.0116 to 0.116	38 hours	Trench and Sandermann, 1978
	Sorbuan	Microsomes	CL L	Tissue concentration	1.6-, 3.6- and 6.12- quinones were predominant metabolites	0.0146	1 hour	Trench and Sandermann, 1980

Table 3.6.2: Toxicoinfection of Higher Plant Cell Cultures (Cont'd)

Chemical	Test Organism	Life Stage or Size	Method	Response Variable	Effect	Exposure Concentration ($\mu\text{g/L}$)	Exposure Duration	Reference
Benzol(a)-pyrene (Cont'd)	Wheat	Actively dividing	CL L	Tissue concentration	80% of cellular label recovered as B[a]P, 5% recovered as actibolites	12.6	48 hours	Haras, 1975
0-Benzolab)-anthracene	Atriplex hortensis	Actively dividing	CL L	Tissue concentration	90% of added label recovered as B[a]A, 5% recovered as actibolites	14.0	48 hours	Haras, 1983
	Chenopodium bonus henricus	Actively dividing	CL L	Tissue concentration	95% of added label recovered as B[a]A, 1% recovered as actibolites	14.0	48 hours	Haras, 1983
	Chenopodium quinoa	Actively dividing	CL L	Tissue concentration	95% of added label recovered as B[a]A, 2% recovered as actibolites	14.0	48 hours	Haras, 1983
	Chenopodium rubrum	Actively dividing	CL L	Tissue concentration	95% of added label recovered as B[a]A, 2% recovered as actibolites	14.0	48 hours	Haras, 1983
Perylene	Atriplex hortensis	Actively dividing	CL L	Tissue concentration	95% of added label recovered as perylene, 1% of added label recovered as actibolites	12.6	48 hours	Haras, 1983

Table 1.6.2. Toxicokinetics of Higher Plant Cell Cultures (Cont'd)

Chemical	Test Organism	Life Stage or Size	Method	Response Variable	Effect	Exposure Concentration ($\mu\text{g/L}$)	Exposure Duration	Reference
Perylene (Cont'd)	<i>Cleopodium bonariensis</i>	Actively dividing	Cl. L.	Tissue concentration	94% of added label recovered as perylene, 5% recovered as metabolites	12.6	48 hours	Haras, 1983
	<i>Cleopodium quinua</i>	Actively dividing	Cl. L.	Tissue concentration	98% of added label recovered as perylene, 1% recovered as metabolites	12.6	48 hours	Haras, 1983
	<i>Cleopodium rubra</i>	Actively dividing	Cl. L.	Tissue concentration	94% of added label recovered as perylene, 5% recovered as metabolites	12.6	48 hours	Haras, 1983

Footnotes: * - Cl. L. = cells in liquid culture; radiolabel

* - B[a]P = benzo[a]pyrene

* - B[a]A = dibenz[ah]anthracene

growth regulator; however, it is difficult to extrapolate these growth effects to phytotoxic effects on whole plants.

Dibenzanthracene and benzo[a]pyrene stimulated growth of carrot cells in culture, and root growth was described as tortuous (Levine 1950). DBA was more active than B[a]P in stimulating growth; 190 mg/L did not result in toxicity or impaired growth.

In general, these studies corroborate the work on laminar algae described in section 2.3.2.1, where cell division was observed to be stimulated by some PAH, and was compared to carcinogenicity in animal systems.

3.6.1.2 Toxicokinetics of Higher Plant Cell Cultures

Use of cultured cells from higher plants to examine the activity of PAH (Table 3.6.2) is useful, in that the experimental material is highly uniform. It is also easy to ensure that the desired dose is delivered to the experimental material, as it is usually suspended in liquid medium. Unfortunately, the artificial cellular organization and lack of higher plant transport systems limit the usefulness of cell cultures for extrapolation to higher plants. None of these studies are primary, as they do not examine whole plants.

Benzo[a]pyrene has been examined most frequently. It is taken up and degraded by plant cells, although the rate of uptake tends to exceed the rate of degradation. Several studies demonstrated up to 90% removal of radiolabeled B[a]P from medium (Trenck and Sandermann, 1978, Harms 1975, Harms et al. 1977, Harms 1983). However, Harms (1975, 1983) demonstrated that in general only a very small percentage (1 - 2%) of the radiolabel may be metabolites of B[a]P; the rest can be extracted as the original compound. The exception was Cheonopodium rubrum, in which almost 60% of the label was associated with extracted residue (e.g. cell wall material): in general, these proportions were species dependent (Harms 1983).

C. rubrum also differed from mungbean, pea, soybean, potato and wheat in that most of the activity was associated with water soluble rather than methanol soluble compounds (Harms 1975). Most of these results are in contrast to the work of Edwards (1986) which demonstrated that most label recovered from bushbean was not anthracene, but metabolites of it. The uptake and metabolism rates are also dependent on the species of PAH; D[ah]A and perylene are taken up to a lesser extent and metabolized less, relative to B[a]P (Harms 1983). For this reason, they would likely be more persistent in the environment.

Trenck and Sandermann (1980) demonstrated that NADPH was not needed for metabolism of B[a]P by plant microsomes, and that quinones were the primary metabolites. These observations indicated that the metabolic pathway of B[a]P by plants may be different from that in the liver, which is NADPH requiring (as a cofactor for B[a]P oxygenation) and has phenolic and dihydrodiols of B[a]P as metabolites. This observation is in contrast to several studies described in section 2.3.2.2.2, which demonstrated that the main metabolites of algal metabolism of B[a]P are dihydrodiols (Lindquist and Warshawsky 1985, Keenan et al. 1984, Warshawsky et al. 1983).

3.7 ECOLOGICAL IMPLICATIONS OF TERRESTRIAL TOXICITY

In view of the limited database, extrapolation of the terrestrial toxicity data to predict ecological implications is somewhat difficult, and predominantly conjecture. Nevertheless, a few generalizations can be made.

Perhaps the most relevant work conducted on mammalian wildlife has been the preliminary assessment of benzo[a]pyrene adduct formation in small mammals (Talmage et al. 1986). While the data is only of a preliminary nature, it is one of the few studies which provides a meaningful end point in relation to an exposure concentration. Demonstration of adducts in vivo is indicative of exposure, uptake and metabolism, and while this is a fundamental relationship, few other studies in this discipline report data of this nature.

The difficulty at this point is determining the biological meaning of a significantly elevated frequency of adducts. During the adult phase, a slightly elevated or even a significantly elevated frequency of adduction may have no repercussions on the individual organism, especially if the effects are somatic and DNA repair mechanisms are efficient. On the other hand, adduct formation with DNA of gametes may pose a serious problem from a population genetics perspective. This may be especially significant in female specimens since genetically damaged ova are not replaced as is the case with spermatozoa in males. Thus attempts to correlate adduct frequency with measures of reproduction may prove useful in PAH wildlife toxicology. Clearly, additional research is required to expand this concept and develop the biological meaning of specific levels of DNA/hemoglobin adducts.

Impacts of PAH and/or petroleum hydrocarbons are quite significant to avian embryos as exemplified by their sensitivity to external applications of only microgram quantities of PAH and/or crude oils. This sensitivity is largely a result of the unusually high basal and inducible activity of embryonic MFO. On the basis of the data presented (section 3.2.2.1) reproductive impacts to avian species are highly likely and warrant further examination. Embryonic exposure may occur during nesting through incidental oil transfer from the parents to the shell, essentially the same route of exposure employed by Bloom and coworkers. In addition, maternal transfer of PAH to developing ova will also facilitate embryonic exposure. While this effect is most likely to occur in shorebirds as a result of aqueous and dietary exposure to PAH, it may also occur in species located near stack emissions or highly urbanized centers.

There is little evidence that algae and higher plants would be detrimentally affected by PAH at expected ambient concentrations. However, there is some evidence that plant species may accumulate PAH in their tissues; this evidence is based upon both experimental and observational data. For herbivores, plants may be a source of dietary exposure to PAH, although not all PAH in plant tissues may be available for digestive absorption.

4. CONCLUSIONS AND RECOMMENDATIONS

4.1 CONCLUSIONS

4.1.1 Aquatic Toxicology

4.1.1.1 General

- 1) Reviews of the aquatic toxicity of PAH have conventionally addressed sublethal effects (especially carcinogenesis) since it was widely regarded that PAH elicited only limited acute toxic impacts (i.e. death) within the confines of their water solubility. The present review, however, emphasizes the significance of photo-induced toxicity in the presence of solar ultraviolet radiation. Through this mechanism, many PAH have been demonstrated to be far more potent than when measured in the absence of photoactivation. It appears this enhanced toxicity is mediated through in vivo creation of free radicals.
- 2) Although more compounds have been demonstrated to be phytotoxic to invertebrates rather than fish, the two group of test organism may effectively be considered equisensitive since the majority of UV-LT50s (LT50s derived during simultaneous exposure to ultra violet light) are less than 48 h at typical concentrations less than 10 ug/L.
- 3) Fish species may be as sensitive, if not more sensitive than invertebrates, to acute lethal concentrations of PAH. This is based on comparison of primary data only.

4.1.1.2 Fish

- 1) Data on acute lethality regarded as "primary" for the purpose of regulation development, was limited to only six compounds (acenaphthene, acridine, anthracene, fluorene, naphthalene and phenanthrene). The 96 h LC50s for conventional PAH toxicity among these six compounds ranged from 0.11 to > 100.0 mg/L (the upper range mediated through solvent carriers). Secondary data exists for an additional eight compounds. Therefore, the acute lethal database for fish is poorly represented in view of the numerous compounds which comprise this class of contaminants.
- 2) Early-life-stage tests indicate that the embryo-larval phase is the most sensitive life stage for the PAH which have been tested under this protocol. Embryonic exposures are useful for the lethality bioassays since they capitalize on mortalities which arise through teratogenic effects. Inferences may also be made on reproductive success.
- 3) Conventional, primary, chronic toxicity data addressing growth and reproduction are limited to naphthalene and acenaphthene. In these cases, growth was a moderate indicator of toxic impacts. However, the acute chronic ratio was relatively small (1.5 to 3.6) indicating a relatively narrow range between conventional chronic and acute toxicity.
- 4) A variety of genotoxicity tests have been effectively used to demonstrate DNA damage caused by metabolites of the model PAH benzo[a]pyrene. Comparative studies on fish using other PAH are generally lacking.

- 5) Use of the lake trout MFO assay may be an effective means of identifying areas suspected to be contaminated with PAH or other MFO inducers.

4.1.1.3 Invertebrates

- 1) Data on acute lethality regarded as primary for the purpose of regulation development exists for thirteen compounds and the EC50 and LC50 ranges from 0.005 to 24.5 mg/L, a range factor of approximately 5000. Secondary data exists for an additional fifteen compounds. Therefore, the primary and secondary acute lethal effects database is better developed for invertebrates than fish.
- 2) Daphnia sp. represents the most frequently tested invertebrate, and is generally the most sensitive invertebrate tested.
- 3) Very limited data exists for subacute and chronic toxicity of PAH to freshwater invertebrates. Adequate information was available for the calculation of only one chronic value which applies to acridine (Chronic Value = 0.57; Acute:Chronic Ratio 3.6). Response variables describing fecundity were the most sensitive descriptors of sublethal chronic impacts for invertebrates.
- 4) Information regarding the presence and activity of mixed function oxidases in freshwater invertebrates is limited. While it is generally assumed that the majority of these organisms have limited MFO capabilities, documentation is lacking. In vitro basal activity of a P-450-like MFO was demonstrated in the freshwater crayfish fish (Cambarus).

- 5) The apparent lack of MFO metabolism and empirical data indicate freshwater invertebrates are significant bioconcentrators of PAH, and therefore should represent an important trophic level at which PAH may be transferred.

4.1.1.4 Plants and Algae

- 1) The valuable studies indicate that alga accumulate PAH in their tissues and therefore represent an added source of contamination in the food chain. This may be mitigated to some extent by the fact that a portion of the accumulated PAH is bound and therefore unavailable.
- 2) The data describing lethal toxicity to algae is poorly addressed in the literature. Effects which have been reported are based on environmentally unrealistic concentrations.
- 3) Photoinduced toxicity has been reported for algae, however conflicting results suggest the scope of effect is less significant than observed in fish and invertebrates.
- 4) De Novo synthesis of PAH in higher plant tissue has been both reported and refuted; it seems that some apparent 'bioaccumulation' may actually be normal tissue constituents.

4.1.2 Terrestrial Toxicology

- 1) Comprehensive toxicity research on terrestrial organisms and PAH is limited to lacking in almost every aspect considered in this review. An exception to this would be the embryotoxicity studies conducted with birds where an established protocol is employed.
- 2) Application of genotoxic tests to discern DNA or hemoglobin adducts are promising, and demonstrate a correlation with ambient soil concentrations of benzo[a]pyrene and the degree of soil contact exhibited by selected wildlife.

- 3) The avian embryo exhibits an active and readily inducible MFO system which accounts for the high sensitivity of embryos to only a few micrograms of selected PAH. This feature also facilitates the use of the avian embryo as a test model for genotoxic studies of PAH.
- 4) In higher plants, PAH are not translocated very far from their site of uptake and are subsequently metabolized. As such, fruits demonstrate very little contamination and plants:soil ratio of PAH content is usually very low.

4.2 Recommendations

- 1) In developing an approach for the environmental regulation of PAH, consideration should be given to incorporation of phototoxicity data. While this data does not easily lend itself to conventional regulatory setting practices, the results, nevertheless, indicate a significant increase in potency which must be addressed in order to ensure ecosystem health.
- 2) In accordance with 1) above, thorough examination of the most common and priority PAH should be undertaken to establish which of these are phototoxic. Follow-up studies to confirm the phototoxic nature would also be desirable.
- 3) Assessment of conventional acute toxicity should include early-life-stages which yield more sensitive data. Tests conducted on fish would appear to be the preferred choice owing to a greater sensitivity as evidenced by the primary data on lethality
- 4) In accordance with 3) above, further development of the toxicity database is warranted, particularly with respect to fish since primary acute exposure data is available for only six compounds and primary chronic exposure data is valuable for only two compounds.

- 5) An investigation into the implications of elevated MFO levels on reproductive hormones and spawning success is warranted, since PAH and other organics known to occur in Ontario waters cause induction of this enzymatic.
- 6) Additional considerations in the regulatory approach are desirable for environments heavily contaminated with both PAH and PCB, since the latter co-contaminant may a) enhance PAH uptake and b) induce the MFO system to metabolize PAH to reactive intermediates.

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APPENDIX

APPENDIX 1

TABLE 1

Guidelines for Evaluation of Aquatic Toxicity Studies
Used for Establishment of Primary Toxicity Data Base and
Suitable for Development of Water Quality Objectives

CATEGORY	GUIDELINE
Primary General	* Adequate control survivability (i.e. <10% mortality). * Acceptable test procedure used and adequately described. * Purified compounds preferred, technical grades considered. * Dilution water of sufficient quality to insure no impact on study (e.g. distilled water not used for controls). * Solvent control used where appropriate. * Proper life stage is used of a species which is preferably found in Ontario, or at least in North America. * Adequate acclimation of test organisms (e.g. minimum 2 weeks for fish). * Measured water concentrations (using acceptable methodology) used in statistical analysis.
Secondary General	* Animals were not fed during an acute study unless cannibalism would occur or if data indicated that feeding did not affect the results. * Concentrations were measured using an acceptable methodology. * Animals were not exposed to stresses other than that being tested (e.g. temperature, pH, oxygen, etc.).

TABLE 1
(Continued)

CATEGORY	GUIDELINE
Secondary General (cont'd)	* Proper life stages were used for each study * Quality of the test water is adequately described (e.g. temperature, oxygen, alkalinity, pH). * Exposure: Fish; preferably flow-through, but static with 24 hour replacement is acceptable. * Preferably <u>no</u> aeration or agitation which may promote volatilization.
Acute Only	* Studies should be conducted for an acceptable period of time (e.g. 48 hours for cladocerans, 96 hours for fish). * Proper life stage is used (e.g. first-instar or cladocerans, juveniles, (<10 g) for fish or otherwise specified). * Partial responses essential (fish: death, invertebrates: death or immobilization). * Acceptable statistical analysis of tolerance <u>not</u> resistance.

TABLE 1 (Continued)

CATEGORY	GUIDELINE
Chronic	* Test conducted using a flow-through monitored system except for cladocerans.
	* Test conditions for cladocerans include solution renewal.
	* Life cycle tests should begin with young less than 48 hours old (24 hours for cladocerans) and should not end until 21 days after the hatching of the next generation except for cladocerans which should be 1 week.
	* Partial life cycle tests should expose individuals at all major life stages (e.g. for fish exposure begins with immature juveniles and continues through to at least 21 days after the hatching of the next generation).
	* Early life stage tests on fish should begin shortly after fertilization and continue to early juvenile development.
	* Appropriate use of multiple comparisons tests in determining significance of effects.
	* Repetition of tests (duplicates for fish, triplicates for cladocerans).

TABLE 2

Guidelines for Evaluation of Phytotoxicity Studies
Used for Establishment of Primary Toxicity Data Base and
Suitable for Development of Air and Soil Quality Objectives

CATEGORY	GUIDELINE
Primary	* Compounds are known; purified or technical * Exposure dose is verified (i.e. applied and received) * Whole plants (multicellular or unicellular) * Growth and exposure conditions are stated and are suitable for plant activity, i.e. $>100 \text{ micromoles m}^{-2}\text{sec}^{-1}$ PAR, $>40\%$ RH, $>15^\circ\text{C}$, adequate nutrients and water * If the conditions are very different from each other, plants are acclimated to exposure facilities for 24 h before exposure * Age and ontogeny of plants are known * Species and cultivar of plants are known * Robust experimental design and valid statistical analysis of data, either multiple range or polynomial regression
Secondary	* Exposure dose and/or compounds not well known, for example ambient collections, or lab studies with unfiltered air or water * Protoplasts * Growth and/or exposure conditions are uncontrolled, or not described, so are not repeatable

TABLE 2 (continued)

CATEGORY	GUIDELINE
Tertiary	* Age or ontogeny not clear
	* Species or cultivar not known
	* Survey type studies; no experimental design or replication, but appropriate statistical analysis
	* Growth and/or exposure conditions suitable for plant activity
	* Invalid data analysis (e.g. no replication of limited experimental study)
Acute	* Mixture dose completely unknown
	* 1-2 day duration; high concentration
	* Well characterized, active life cycle
	* Growth as well as injury responses
	* Sacrifice is temporally close to exposure
	* Limited mortality in treated plants
Chronic	* Exposure conditions may be somewhat marginal for plant activity
	* Several weeks; low concentration
	* Exposure is initiated at early life stage, prior to or at exponential growth phase
	* Concentration is monitored and maintained
	* Growth as well as injury responses
	* Harvest can be farther from exposure time

TABLE 2 (continued)

CATEGORY	GUIDELINE
	* No mortality in treated plants * Exposure conditions must be suitable for plant activity

